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FACTORS AFFECTING THE GERMINATION OF VARIOUS DROPSEED GRASSES (SPOROBOLUS SPP.) [1]

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INTRODUCTION

At the request of the Soil Conservation Service, United States Department of Agriculture, a study of the optimum conditions for the germination of the seed of *Sporobolus* spp. was begun in 1935. Hoover (*22*) [3] states that these grasses can be used widely in erosion-control work because of their adaptation to the climatic and soil conditions of the Great Plains and the Southwest. In addition to the practical importance of the germination behavior of the seed of these plants, the material furnished an opportunity to study the diverse behavior of several species of a genus with an unusual and variable fruit structure. With some species there were enough samples to study the variability and differences in response to various treatments within the species.

EARLIER INVESTIGATIONS

Guérin (*16*) reported that the strongly developed pericarp of *Sporobolus tenacissimus*, of *S. macrospermus*, and of *S. ciliatus* presents a very specialized structure in which almost the entire pericarp is transformed into mucilage of such a nature that under the influence of the least trace of water the seed is liberated. In *S. heterolepsis* the outer layers of the fruit coat are strongly sclerified, and only the inner zone is composed of mucilage. The seed coat in *Sporobolus* is considerably developed.

Jackson (*23*) stated that the seed coat of *Sporobolus* prevents water absorption, but no data were presented in support of the statement. She germinated the seed at constant temperatures of 25° or 35° C. on blotting paper in Petri dishes or in sandy loam. She found that shaking the seed with sand from 4 to 9 hours was of no benefit.

Blake (*4*) reported that seed of *Sporobolus asper* varied in response from year to year. She stated that the germination of the seed improved with age under the conditions she used.

Wilson (*38*), working with *Sporobolus strictus*, *S. cryptandrus*, *S. flexuosus*, *S. wrightii*, and *S. airoides*, found that with the exception of *S. airoides* the seeds did not germinate at all well without scarifying. He stated that the seed coats were almost impervious to water. He obtained over a 6-year period an average germination of 3.75 percent

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[3] Italic numbers in parentheses refer to Literature Cited, p. 714.

for *S. cryptandrus*, 1.11 percent for *S. flexuosus*, 1.54 percent for *S. strictus*, 13.17 percent for *S. wrightii*, and 35.69 percent for *S. airoides*. The germination tests were made at a temperature alternation of 20° to 30° C.

The extensive literature on dormancy and on the physiology of germination will not be reviewed here, but pertinent papers will be mentioned in the discussion of results.

MATERIAL AND METHODS

The following species were included in this study: *Sporobolus cryptandrus* (Torr.) A. Gray, sand dropseed; *S. flexuosus* (Thurb.) Rydb., mesa dropseed; *S. contractus* Hitchc., spike dropseed; *S. giganteus* Nash, giant dropseed; *S. airoides* (Torr.) Torr., alkali sacaton; *S. wrightii* Munro., sacaton; and *S. asper* (Michx.) Kunth. The seed was furnished by M. M. Hoover of the Soil Conservation Service from various regional plantings and from collections of native plants, and by the Division of Forage Crops and Diseases, Bureau of Plant Industry. Also one sample (No. 769232) was collected from wild plants by Mary Thayer, of the Seed Laboratory, Kansas State College of Agriculture and Applied Science. The origin, date of collection, and other historical data on the samples are given in table 1

After receipt, the seed was stored in the laboratory at room temperature. The seed as received was cleaned by means of an air-blast blower and sieves. Only fully developed caryopses were used in the study, but in these were sometimes included seed of varying sizes. The "seed" as referred to in this paper includes the true seed with attached pericarp and, in some cases, with the lemma and palea. The seed was put to germinate in Petri dishes on paper toweling moistened with tap water or with a 0.2-percent solution of potassium nitrate, at various constant and alternating temperatures. The alternation of temperatures was secured by transferring the test from one chamber to another. The test was at the first temperature listed for 17 hours, and at the last temperature for 7 hours daily. In order to obtain light in combination with a high temperature an alternation of room temperature to 35° C. was used. The tests were placed in the north window in an air-conditioned room from 4 p. m. until 9 a. m. and at 35° from 9 a. m. until 4 p. m. The room temperature varied between 16° and 24° but was maintained at near 20° much of the time except during the summer. The 20° to 30° alternation, with light exposure, was obtained by using a specially built daylight germinator for the 30° temperature.

To prechill, the seed was placed on the moistened paper toweling in the Petri dishes and held at 3° C. for the designated number of days prior to being placed at the germination temperature. The temperature of the 3° chamber varied between 2° and 5°. The date of counts was calculated from the day the seed was placed to prechill.

For sulfuric acid treatments, the seed was placed in a small porcelain crucible, covered with an excess of the acid, and stirred constantly for various lengths of time, after which it was washed in running tap water for 30 to 45 minutes and dried thoroughly before being tested for germination. The concentrated sulfuric acid used had a specific gravity of 1.84 and was of at least 94-percent concentration. Sulfuric acid approximately 71 percent by volume (approximately 80

TABLE 1.—*Historical data on all samples of seed of Sporobolus spp.*

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Sample No.	Senders' identification No.	Approximate purity	Species	Place of collection	Year or date of collection	Date received	Notes by collector
		Percent					
757544	Wy341-251	80	*Sporobolus cryptandrus*	Cheyenne, Wyo		Mar. 23, 1935	
101	101	98	*Sporobolus cryptandrus*	Evans, Colo	Oct. 1, 1938	Nov. 12, 1938	Sandy soil.
766904		97	*Sporobolus cryptandrus*	Amarillo, Tex	Sept. and Oct. 1937	Dec. 17, 1937	From vacant lots.
100	100	98	*Sporobolus cryptandrus*	do	Sept. 19, 1938	Nov. 5, 1938	From same lots as 766904.
1256	1256	98	*Sporobolus cryptandrus*	Perryton, Tex	Aug. 1938	Nov. 12, 1938	From high plains soils on abandoned farmsteads.
272727		93	*Sporobolus cryptandrus*	O'Neill, Nebr	1935	Mar. 31, 1938	
769223	A810	93	*Sporobolus cryptandrus* [1]	Tucson, Ariz	June 1937	Aug. 24, 1938	Grown under irrigation.
A810	A810	98	*Sporobolus cryptandrus* [1]	do	1938	Feb. 7, 1939	Nursery stock.
766903	Okla. 1605	93	*Sporobolus cryptandrus*	Seiling, Okla	Aug. 1937	Dec. 17, 1937	
01949	O-1949	98	*Sporobolus cryptandrus*	do	Sept. 20, 1938	Nov. 12, 1938	Abandoned fields and along roadways.
272728		98	*Sporobolus cryptandrus*	do	1936	Mar. 31, 1938	
272726		99	*Sporobolus cryptandrus*	Pullman, Wash	1935	do	
767694	52	93	*Sporobolus cryptandrus*	Morgan, Utah	Jan. 1938	Feb. 23, 1938	
769232		82	*Sporobolus cryptandrus*	Manhattan, Kans	Aug. 18, 1938	Aug. 26, 1938	Vacant lot.
767742	55	75	*Sporobolus contractus*	Morgan, Utah	Jan. 1938	Feb. 23, 1938	
767287	A810	84	*Sporobolus contractus*	Tucson, Ariz	June 1937	Jan. 10, 1938	Nursery stock.
A8763	A8763	98	*Sporobolus contractus*	do	Jan. 26, 1939	Feb. 7, 1939	Roadside near nursery. Seeds matured in late summer. Stood on plants till collected.
A8392	A8392	98	*Sporobolus giganteus*	do	Oct. 21, 1938	do	Nursery stock.
767281	A923	74	*Sporobolus giganteus*	Willcox, Ariz	1934	Jan. 10, 1938	
767283	A8081	36	*Sporobolus flexuosus* [2]	do	Oct. 1936	do	
A8081	A8081	98	*Sporobolus flexuosus* [2]	Tucson, Ariz	Sept. and Oct. 1938	Feb. 7, 1939	Nursery stock.
767280	A10117	55	*Sporobolus flexuosus* [2]	Willcox, Ariz	Nov. 20, 1937	Jan. 10, 1938	
767711	16	64	*Sporobolus flexuosus* [2]	Leeds, Utah	Feb. 11, 1938	Feb. 23, 1938	
767741	53	90	*Sporobolus flexuosus* [2]	Morgan, Utah	Jan. 1938	do	
767716	21	69	*Sporobolus airoides*	Leeds, Utah	Feb. 11, 1938	do	
767700	3-U, No. 2	86	*Sporobolus airoides*	Price, Utah	Jan. 24, 1938	do	
272724		97	*Sporoboule airoides*	Southcentral Kansas	1936	Mar. 31, 1938	
A8501	A8501	98	*Sporobolus airoides*	Winslow, Ariz	Nov. 1936	Feb. 7, 1939	
769224	A666	79	*Sporobolus wrightii*	Tucson, Ariz	Nov. 1937	Aug. 24, 1938	Grown under irrigation.
A666	A666	98	*Sporobolus wrightii*	do	Aug. 1938	Feb. 7, 1939	Nursery stock.
272725		89	*Sporobolus asper*	do	1937	Mar. 31, 1938	

[1] Probably a mixture; some seeds smooth, like *S. cryptandrus*, most of them striate, like *S. contractus*.
[2] Not very sure of identification of this group. Apparently contains some *S. cryptandrus*.

percent by weight) was secured by diluting 3 volumes of acid with 1 volume of distilled water.

The sprouted seeds were counted on the third, fifth, and seventh days after being placed at germination temperatures and at 7-day intervals thereafter. It was necessary to count the sprouts often during the first week, especially in tests with treated seed, because germination was rapid under proper conditions. Seeds that produced a normally developed root and shoot were considered as germinated.

The germination values summarized in the various tables are means based on duplicate tests of 100 seeds each. In tables 2, 5, 6, 14, 15, and 16, half percents have been raised to the next higher percentages. In tables 3, 4, 8, and 11, value for error and tests of significance have been calculated by the analysis-of-variance method as adapted by Snedecor (*31*). Differences have been designated significant when the probability is equal to or less than 5 percent and highly significant when equal to or less than 1 percent. Mean squares marked with an asterisk (*) are significant with reference to error and marked with a double asterisk (**) are highly significant with reference to error.

EXPERIMENTAL RESULTS

SPOROBOLUS CRYPTANDRUS

Germination of Intact Seeds

Five samples of seed of *Sporobolus cryptandrus* were tested at a wide range of constant and alternating temperatures, in some cases with definite light exposure, in order to determine the general response to temperature and light. The effect of potassium nitrate in the germination medium was also determined. Results are given in table 2. In general, germination was best at the room temperature to 35° C. alternation, in which the seed received some light from a north window while at a room temperature varying around 20°. When the seed had no pretreatment, room temperature to 35° alternation, with one exception, was definitely superior to any combination that included a temperature continuously above 20° for the longer period or above 35° for the shorter period of the alternation. Germination at 15° to 35° alternation was somewhat better than at 20° to 35°, and at either of these temperatures it was much superior to 20° to 30° alternation with light. Constant temperatures produced negligible results, but 35° constant was superior to 10°, 15°, 20°, 25°, or 30°. Potassium nitrate was beneficial, especially at the more favorable temperatures.

In order to determine more definitely the effect of light at room temperature to 35° C. alternation during May 1936, a check test of sample No. 757544 was placed in a tin box to exclude all light. (Results are not given in tabular form.) Germination with light excluded was 15 percent with nitrate and 10 percent with water, compared with 67 percent with nitrate and 31 percent with water on the test exposed to light. The results at room temperature to 35° with light excluded were comparable to those at 20° to 35° (24 percent with nitrate and 8 percent with water).

With some samples a much higher germination percentage was obtained after the seed had been prechilled at 3° C. A more detailed study was made of the effect of prechilling, the results of which will be presented later. Results not given in the tables indicated that storing

TABLE 2.—*Germination in 49 to 56 days of five samples of seed of Sporobolus cryptandrus at various temperatures*

Prechilling period (days)	Germination temperature (° C.)	Germination in tests begun on indicated date with specified treatment of sample No.—													
		757544						766903		766904		769223		769232	
		May 10, 1935		Nov. 22, 1935		March 26, 1936		Jan. 13, 1938		Jan. 19, 1938		Sept. 7, 1938		Sept. 12, 1938	
		Potassium nitrate	Water	Potassium nitrate	Water	Potassium nitrate	Water	Potassium nitrate	Water	Potassium nitrate	Water	Potassium nitrate	Water	Potassium nitrate	Water
		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
0	10	0	0												
0	15	1	1												
0	20	1	0												
0	25	0	0												
0	30	3	1												
0	35	21	3												
0	10 to 25	11	2												
0	15 to 25	4	2												
0	10 to room	2	1												
0	10 to 30	25	4												
0	10 to 35			55	25										
0	15 to 35			77	49	62	34	77	42	19	12				
0	25 to 15	2	0												
0	35 to 10	11	6												
0	20 to 30 with light.	2	1	27	13	10	9	33	16	3	1	5	2	20	6
0	20 to 35	9	13	43	18	10	4	67	26	10	5	16	10	78	27
0	Room to 35	54	29	70	43	93	76	93	56	61	12	13	7	87	25
0	20 to 40							66	40	16	5	19	6	70	23
0	25 to 40							57	28	7	3				
7	20 to 35			42	28	16	7	90	72	24	17				
7	Room to 35			50	18	88	76	97	83	51	16				
7	15 to 35							85	77	25	10				
14	20 to 35			68	41	43	12	97	83	36	13	70	51	86	59
14	Room to 35	69	27	80	46	97	79	92	75	50	19	75	51	90	57
14	15 to 35							93	86	52	31				
21	20 to 35			81	70	45	40	93	81	44	13				
21	Room to 35	72	35	68	25	91	85	92	79	55	17				
21	15 to 35							80	84	48	22				
28	20 to 35			99	85	80	77	87	82	54	27	85	88	86	58
28	Room to 35	85	63	98	73	96	86	97	86	38	25	87	85	88	67
28	20 to 30			44	17	51	36								
28	30			63	33	34	34								
28	35			68	63	69	73								
28	15 to 35							87	84	53	28				
56	20 to 35			88	86	96	99								
56	Room to 35			93	70	90	87								

the seed dry at 2° did not have the effect obtained by prechilling moist seed at 3°.

Although with some samples practically complete germination of the viable seeds was obtained at room temperature to 35° C. alternation when the substratum was moistened with potassium nitrate, other samples appeared to be resistant to the combined effects of temperature, light, and nitrate and to require additional treatment. In later work on pretreatment of the seed, room temperature to 35° alternation was used as the standard temperature, but the 20° to 35° alternation was continued as a check, since it approximated the same temperature range with a better control of the lower temperature.

TREATMENT WITH SULFURIC ACID

In order to determine the possible effect of the seed covering in restricting germination, seed was treated with sulfuric acid. Preliminary trials with concentrated sulfuric acid (94 percent) caused

injury to the seed, and germination results were low. Untreated seed and seed treated for 1, 2, 5, 7, and 9 minutes with approximately 71-percent sulfuric acid were germinated with potassium nitrate and with water at room temperature to 35° C. alternation and at 20° to 35° alternation. The germination results with 8 samples, untreated and treated for 2 and 5 minutes, are given in table 3.

In this, as well as in subsequent tables, the variance for duplicates was very low in comparison with the variance for sample interaction, indicating uniformity of conditions of germination.

There was a very highly significant difference between the mean results for the two germination-temperature treatments and between potassium nitrate and water. Pretreatment with approximately 71-percent sulfuric acid overcame the resistance to germination. A 5-minute acid treatment was significantly better than a 2-minute acid treatment. In general, however, treatment for 2 minutes was sufficient when followed by germination at room temperature to 35° C. alternation with the use of potassium nitrate. This length of treatment was not injurious even to those samples that germinated well without treatment. Sample No. 272727 required a longer treatment (5 or even 7 minutes) for maximum germination. Five-minute treatment caused injury to sample No. O–1949, which germinated well without treatment at the optimum conditions. In all cases tried, treatment for 9 minutes was injurious. The highly significant interaction between the germination temperatures and the acid treatments is brought about by the very low results for the untreated seed at the less favorable temperature. In a few trials at the optimum condition a 1-minute acid treatment was almost as good, or as good, as the 2-minute treatment.

TABLE 3.—*Germination in 28 days of seed of Sporobolus cryptandrus at 2 temperature alternations after treatment with approximately 71-percent sulfuric acid, November 1938*

ORIGINAL DATA

Treatment of substratum and sample No.	Observations	Germination after acid treatment for indicated period at—							
		Room temperature to 35° C.				20° to 35° C.			
		0 minute	2 minutes	5 minutes	Mean	0 minute	2 minutes	5 minutes	Mean
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
766904	2	67.5	97.5	94.0	86.33	10.5	95.0	89.0	64.83
100	2	53.5	95.0	99.5	82.67	5.5	75.5	94.5	58.50
1256	2	55.5	97.5	89.0	80.67	20.0	90.0	87.5	65.83
272727	2	54.5	63.5	85.5	67.83	5.0	57.5	61.0	41.17
769223	2	30.0	78.5	78.5	62.33	15.5	52.0	81.5	49.67
101	2	90.0	96.5	99.0	95.17	6.5	76.0	95.0	59.17
769232	2	91.0	91.5	89.0	90.50	87.0	91.5	86.5	88.33
O–1949	2	98.0	99.5	78.0	91.83	62.0	93.5	68.0	74.50
Total or mean	16	67.50	89.93	89.06	82.16	26.50	78.87	82.87	62.75
Water:									
766904	2	27.5	87.5	90.5	68.50	9.0	79.5	87.0	58.50
100	2	9.5	44.0	85.5	46.33	2.5	32.5	47.0	27.33
1256	2	21.5	86.0	86.0	64.50	5.0	65.0	68.5	46.16
272727	2	27.5	22.0	48.5	32.67	0.5	14.5	37.5	17.50
769223	2	16.0	50.5	78.5	48.33	14.5	31.5	69.5	38.50
101	2	68.5	71.5	84.5	74.83	3.0	39.5	66.5	36.33
769232	2	48.5	75.0	71.5	65.00	15.0	76.0	72.0	54.33
O–1949	2	65.5	97.0	73.0	78.50	17.5	86.0	86.0	56.50
Total or mean	16	35.56	66.69	77.25	59.83	8.37	53.06	64.25	41.89
Grand total or mean	32	51.53	78.31	83.15	70.99	17.43	65.96	73.56	52.32

TABLE 3.—*Germination in 28 days of seed of Sporobolus cryptandrus at 2 temperature alternations after treatment with approximately 71-percent sulfuric acid, November 1938*—Continued

ORIGINAL DATA—Continued

Treatment of substratum or germination temperatures	Total observations	Mean germination after acid treatment for—			
		0 minutes	2 minutes	5 minutes	Mean
	Number	*Percent*	*Percent*	*Percent*	*Percent*
Potassium nitrate	32	47.00	84.40	85.96	72.45
Water	32	21.96	59.87	70.75	50.86
Room temperature to 35° C	32	51.53	78.31	83.15	70.99
20° to 35° C	32	17.43	65.96	73.56	52.32
All treatments	64	34.48	72.13	78.35	61.65

ANALYSIS OF VARIANCE FOR ALL TREATMENTS

Source	D. F.	Mean square	*F*
Between samples	7	3,869.04	
A (2 v. 5 minutes)	1	1,237.53	2.65
B (2+5 minutes v. twice 0 minutes)	1	70,905.01	**152.15
C (KNO_3 v. H_2O)	1	22,381.92	**48.03
D (temperature$_1$ v. temperature$_2$)	1	16,744.00	**35.93
B×D	1	5,704.17	**12.24
Other interactions	6	335.48	
Error (sample × treatments)	77	466.01	
Between duplicates	96	26.39	
Total	191		

ANALYSIS OF VARIANCE FOR 2- AND 5-MINUTE ACID TREATMENTS

Between samples	7	2,773.68	
A (2 v. 5 minutes)	1	1,237.53	*4.10
C′ (KNO_3 v. H_2O)	1	12,640.50	**41.92
D′ (temperature$_1$ v. temperature$_2$)	1	3,850.03	**12.77
Interactions	4	241.54	
Error (sample × treatments)	49	301.55	
Between duplicates	64	26.53	
Total	127		

COMPARISON OF PRECHILLING AND ACID TREATMENTS

The germination results of eight samples after prechilling for 14, 28, and 56 days at 3° C. and after a 2-minute treatment with approximately 71-percent sulfuric acid are given in table 4. Prechilling for 28 or 56 days was superior to prechilling for 14 days. Taken as a whole, the 2-minute treatment with acid was significantly superior to the prechilling treatments. With either prechilling or acid treatment, the use of potassium nitrtate gave increased germination.

SIZE OF SEED AND GERMINATION

The cleaned seed of sample No. 769223 was quite variable in size. On September 7, 1938, a portion of the sample was passed through sieves, and the different seed sizes were germinated separately. The results are given in table 5. The results after prechilling represent the full value of the sample for the different groups as the seed either germinated or decayed. The smaller seed had less potential viability than the larger and was benefited less by prechilling, indicating a less resistant seed coat. This was the only sample of *Sporobolus cryptandrus* studied that did not have a potential germination value of more

than 90 percent. When the smaller seed had been sieved out, the germination value of the remainder of the sample was above 90 percent.

TABLE 4.—*Germination in 84 days with room temperature to 35° C. alternation of eight lots of Sporobolus cryptandrus seed prechilled at 3° for various periods or treated with approximately 71-percent sulfuric acid for 2 minutes, November 1938*

ORIGINAL DATA

Sample No.	Observations	Germination of seed with potassium nitrate after indicated treatment					Germination of seed with water after indicated treatment				
		Prechilled for—			Acid-treated for 2 minutes	Mean	Prechilled for—			Acid-treated for 2 minutes	Mean
		14 days	28 days	56 days			14 days	28 days	56 days		
	Number	*Per-cent*	*Per-cent*	*Per-cent*	*Percent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Percent*	*Per-cent*
766904	2	86.5	82.5	82.0	97.5	87.12	65.5	78.5	80.0	87.5	77.87
100	2	36.0	56.5	63.5	95.0	62.75	10.0	13.0	21.0	44.5	22.12
1256	2	62.5	81.0	86.5	97.5	81.87	31.0	42.5	61.5	86.0	55.25
272727	2	74.0	71.5	96.0	67.5	77.25	57.0	62.0	79.0	23.5	55.37
769223	2	83.0	85.5	74.5	80.5	80.87	45.0	89.0	87.5	62.5	71.00
101	2	81.5	87.0	74.0	96.5	84.75	44.0	56.0	29.0	71.5	50.12
769232	2	91.0	90.5	93.0	92.0	91.62	85.0	85.0	84.0	75.5	82.37
O–1949	2	92.0	100.0	90.5	99.5	95.50	50.0	81.5	71.0	97.0	74.87
Total or mean	16	75.81	81.81	82.50	90.75	82.72	48.44	63.44	64.12	68.50	61.12

Treatment of substratum	Total observations	Mean germination for all samples at indicated treatment				
		Prechilled for—			Acid-treated for 2 minutes	
		14 days	28 days	56 days		
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Potassium nitrate and water	32	62.12	72.62	73.31	79.62	71.91

ANALYSIS OF VARIANCE FOR ALL TREATMENTS

Source	D. F.	Mean square	*F*
Between samples	7	3,345.23	
A (KNO_3 v. H_2O)	1	14,921.28	**34.34
B (Prechilling v. acid)	1	2,531.76	*5.83
C (14 v. 56 days prechilling)	1	2,002.56	*4.61
D (14+56 v. (2) (28) days prechilling)	1	513.52	1.18
Treatment interaction	3	145.53	
Error (sample×treatment)	49	434.45	
Between duplicates	64	28.95	
Total	127		

ANALYSIS OF VARIANCE FOR PRECHILLED TREATMENTS

Between samples	7	3,573.32	
A (KNO_3 v. H_2O)	1	10,965.37	**39.94
C′ (14 v. 56 days prechilling)	1	2,002.56	*7.29
D′ (14+56 v. (2) (28) days prechilling)	1	513.52	1.87
Treatment interactions	2	216.00	
Error (sample×treatment)	35	274.58	
Between duplicates	48	26.19	
Total	95		

TABLE 5.—*Germination in 49 days of seed of Sporobolus cryptandrus, sample 769223, grouped according to seed size, September 1938*

Sieve size (mm.)[1]	Germination of seed at room to 35° C. temperature alternation			
	Not prechilled		Prechilled 28 days	
	Germinated with potassium nitrate	Germinated with water	Germinated with potassium nitrate	Germinated with water
	Percent	*Percent*	*Percent*	*Percent*
1.016	25	3	97	98
0.686	35	12	91	98
0.610	32	3	84	89
0.508	24	18	54	45
Mean	29.0	9.0	81.5	82.5

[1] The seed passed through the indicated sieve size and was retained by the next smaller.

REGIONAL OR SAMPLE DIFFERENCE

There were differences in the degree of resistance to germination among the different samples of *Sporobolus cryptandrus* studied. Samples from the same State were of similar behavior. Samples from Texas, Arizona, Nebraska, and Wyoming did not give maximum germination at room temperature to 35° C. alternation with light and nitrate, whereas samples from Colorado, Oklahoma, Washington, Utah, and Kansas gave maximum germination under these conditions (table 6). All *S. cryptandrus* samples studied (except No. 769223) had a potential germination value of 90 percent or above. The States furnishing samples of seed having similar behavior do not form regional groups; however, it is believed that the resistant samples came from localities with semiarid conditions. Samples that germinated promptly at room temperature to 35° alternation with nitrate are referred to in this paper as "nonresistant," and samples that required additional treatment as "resistant."

TABLE 6.—*Germination in 49 days of Sporobolus cryptandrus seed of various ages collected in different States*

Sample No.	State where sample was collected	Date of germination test	Approximate age of seed	Germination, with indicated treatment, at—					
				20° to 35° C.		Room to 35° C.		Room temperature to 35° C. (after prechilling at 3° for 28 days)	
				Potassium nitrate	Water	Potassium nitrate	Water	Potassium nitrate	Water
			Months	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
766904	Texas	Jan. 18, 1938	3	9	5	61	12	37	25
		July 26, 1938	9	20	6	15	6	----	----
		Nov. 15, 1938	13	11	10	68	28	83	78
100	do	Nov. 16, 1938	2	7	3	54	10	55	12
1256	do	Nov. 18, 1938	3	21	5	56	22	71	41
272727	Nebraska	Apr. 16, 1938	[1] 29	4	1	33	4	----	----
		July 29, 1938	[1] 32	6	1	1	0	----	----
		Nov. 10, 1938	[1] 36	5	1	55	28	71	62
		Feb. 15, 1939	[1] 39	5	0	61	25	----	----

See footnotes at end of table.

TABLE 6.—*Germination in 49 days of Sporobolus cryptandrus seed of various ages collected in different States*—Continued

Sample No.	State where sample was collected	Date of germination test	Approximate age of seed	Germination, with indicated treatment, at—					
				20° to 35° C.		Room to 35° C.		Room temperature to 35° C. (after prechilling at 3° for 28 days)	
				Potassium nitrate	Water	Potassium nitrate	Water	Potassium nitrate	Water
			Months	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
769223	Arizona	Sept. 7, 1938	15	16	10	13	7	87	85
		Nov. 9, 1938	17	16	15	33	16	85	89
		Feb. 16, 1939	20	27	15	76	26		
A810	do	Mar. 3, 1939	[1] 14	27	14	59	20	66	50
757544	Wyoming	May 10, 1935	[2] 7	9	13	54	29	85	63
		Nov. 22, 1935	[2] 13	43	18	70	43	98	73
		Mar. 26, 1936	[2] 17	10	4	90	76	97	85
		May 15, 1936	[2] 19	23	8	67	31	76	42
		Sept. 15, 1937	[2] 35	49	21	89	72	88	83
		July 26, 1938	[2] 45	28	16	14	8	56	32
		Nov. 8, 1938	[2] 49	32	22	95	83		
101	Colorado	Nov. 18, 1938	1	9	4	91	69	76	35
776903	Oklahoma	Jan. 18, 1938	5	67	26	92	56	97	86
		July 25, 1938	11	62	19	68	33		
		Nov. 8, 1938	15	61	33	95	56		
O–1914	do	Nov. 21, 1938	2	64	20	99	66	100	77
272728	do	Apr. 16, 1938	[1] 18	80	72	90	65	93	74
		July 29, 1938	[1] 21	65	56	73	54		
		Nov. 14, 1938	[1] 25	63	56	98	85	95	96
272726	Washington	Apr. 16, 1938	[1] 29	64	62	98	95	97	94
		July 28, 1938	[1] 32	82	79	82	73		
		Nov. 8, 1938	[1] 36	70	68	97	98		
767694	Utah	Mar. 7, 1938	1	57	25	96	78	96	97
		July 28, 1938	5	83	45	75	34		
		Nov. 8, 1938	9	85	42	97	83		
769232	Kansas	Sept. 12, 1938	1	78	27	87	25	88	67
		Nov. 9, 1938	2	87	17	92	55	92	84

[1] Actual date of collection not known.
[2] Based on assumption that seed was collected in fall of 1934.

CHANGE WITH AGE

In general, germination was higher for all samples at room temperature to 35° C. alternation than at 20° to 35° whatever the age of the seed when tested (table 6). The beneficial effect of light during germination was observed for seeds up to at least 3 years old. A consistent change of germination with age of the seed was not evident when it was germinated at 20° to 35° alternation. At room temperature to 35° alternation seed germination was very low during the summer months, as the room temperature was more nearly 25° than 20° during this period. Except in these summer tests, there was a general increase of germination at room temperature to 35° alternation as the seed became older up to 4 years, when the experiment was terminated. The nonresistant samples that germinated well at the first test at room temperature to 35° alternation with potassium nitrate showed increased germination with age of seed when moistened with water. With the exception of one sample that gave practically complete germination with water, the use of potassium nitrate increased germination markedly for seed of all ages. A combination of both light and potassium nitrate was essential for maximum germination. Tests that had been prechilled at 3° for 28 days and then placed at room

temperature to 35° alternation tended to show better germination as the seed became older; the difference was most striking when water was used to moisten the substratum.

In the foregoing experiments the tests remained in the germinator for a long period to give full opportunity for all germination possible. However, very little germination occurred after 21 days for either treated or untreated seed, and in some cases there was little germination after 7 or 14 days.

SPOROBOLUS FLEXUOSUS

Germination of Intact Seeds

The effect of the constant temperatures 20°, 25°, 30°, and 35° C. and of the temperature alternations 15° to 25°, 20° to 30° with light, 10° to 35°, 15° to 35°, 20° to 35°, room temperature to 35°, 20° to 40°, 25° to 40°, and 35° to 10° was determined on the germination of fresh and older seed of *Sporobolus flexuosus* in February 1938. The seed was germinated with potassium nitrate and with water. The seed germinated very poorly and practically in the same proportion at all temperatures, and nitrate and light appeared to have no effect. The highest germination obtained was 15 percent. The results are not given in tabular form.

Treatment with Sulfuric Acid

On November 29, 1938, seed of four samples of *Sporobolus flexuosus*, untreated and treated with approximately 71-percent sulfuric acid for 4 minutes, was germinated at room temperature to 35° C. alternation and at 20° to 35° with the use of potassium nitrate and of tap water. The results of the germination tests are given in table 7. In every case germination of untreated seed was less than half the mean germination of untreated seed and seed treated 4 minutes, indicating a significant effect of acid treatment for 4 minutes. The use of potassium nitrate was not beneficial. Results at the two germination temperatures were similar.

On February 20, 1939, three of these samples of *S. flexuosus*, untreated and treated with sulfuric acid for 4 and 7 minutes, were germinated under the same conditions as in the November test. The results of the germination tests are given in table 8. Maximum germination was obtained in 21 days. A 4-minute treatment was definitely superior to no treatment and to a 7-minute treatment. A 7-minute treatment appeared to injure some of the seeds. A few tests with a 2-minute treatment indicated that a longer period of treatment was necessary. There was no difference in the results at the two germination conditions, indicating that the light received at room temperature was of no benefit. There was an indication of a slight benefit from the use of potassium nitrate.

After transformation of the original data of table 8 to angular values by the use of table 12 of Fisher and Yates (*13*), an analysis of variance and tests for significance did not indicate any different conclusions from those based on the original values.

The intact seed of the three samples tested in November 1938 and again in February 1939 showed no afterripening effect in 3 months' time.

TABLE 7.—*Germination in 56 days of seed of Sporobolus flexuosus at two temperature alternations, with and without treatment with approximately 71-percent sulfuric acid, Nov. 29, 1938*

Treatment of substratum and sample No.	Observations	Germination after indicated period of acid treatment at—					
		Room temperature to 35° C.			20° to 35° C.		
		0 minute	4 minutes	Mean	0 minute	4 minutes	Mean
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
767711	2	20.0	63.0	41.50	13.5	58.0	35.75
767741	2	5.5	59.5	32.50	6.0	57.5	31.75
767280	2	6.0	95.5	50.75	5.0	94.5	49.75
767283	2	9.5	88.0	48.75	9.5	80.5	45.00
Total or mean	8	10.25	76.50	43.37	8.50	72.62	40.56
Water:							
767711	2	11.5	52.5	32.00	7.0	51.0	29.00
767741	2	11.5	51.5	31.50	7.0	58.5	32.75
767280	2	5.5	94.5	50.00	6.0	94.5	50.25
767283	2	6.5	82.0	44.25	9.0	87.0	48.00
Total or mean	8	8.75	70.12	39.43	7.25	72.75	40.00
Total or mean	16	9.50	73.31	41.40	7.87	72.68	40.28

Treatment of substratum	Total observations	Mean germination for the 2 temperature alternations		
		0 minute	4 minutes	Mean
	Number	*Percent*	*Percent*	*Percent*
Potassium nitrate	16	9.37	74.56	41.96
Water	16	8.00	71.43	39.71
Potassium nitrate and water	32	8.68	72.99	40.84

TABLE 8.—*Germination in 21 days of seed of Sporobolus flexuosus at two temperature alternations, with and without 71-percent sulfuric acid treatments, Feb. 20, 1939*

ORIGINAL DATA FOR ROOM TEMPERATURE TO 35° C.

Treatment of substratum and sample No.	Observations	Germination after acid treatment for—			
		0 minute	4 minutes	7 minutes	Mean
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
767711	2	15.0	84.0	64.0	54.33
767741	2	6.0	66.5	66.0	46.16
767283	2	3.0	89.5	58.5	50.33
Total or mean	6	8.0	80.0	62.83	50.27
Water:					
767711	2	9.0	76.0	60.5	48.50
767741	2	4.5	60.5	57.5	40.83
767283	2	6.0	83.5	51.0	46.83
Total or mean	6	6.5	73.33	56.33	45.38
Total or mean	12	7.25	76.67	59.58	47.83

TABLE 8.—*Germination in 21 days of seed of Sporobolus flexuosus at two temperature alternations, with and without 71-percent sulfuric acid treatments, Feb. 20, 1939*—Continued

ORIGINAL DATA FOR 20° TO 35° C.

Treatment of substratum and sample No.	Observations	Germination after acid treatment for—			
		0 minute	4 minutes	7 minutes	Mean
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
767711	2	8.5	77.5	52.5	46.16
767741	2	7.0	71.0	61.5	46.50
767283	2	7.5	88.5	55.0	50.33
Total or mean	6	7.67	79.0	56.33	47.66
Water:					
767711	2	9.0	71.5	45.5	42.00
767741	2	4.5	67.5	51.5	41.17
767283	2	6.0	85.0	39.0	43.33
Total or mean	6	6.50	74.66	45.33	42.17
Total or mean	12	7.08	76.83	50.83	44.91

MEAN FOR THE 2 TEMPERATURE ALTERNATIONS

Potassium nitrate	12	7.83	79.50	59.58	48.96
Water	12	6.50	73.99	50.83	43.77
Water and potassium nitrate	24	7.16	76.74	55.20	46.36

ANALYSIS OF VARIANCE FOR ALL TREATMENTS

Source	D. F.	Mean square	*F*
Between samples	2	132.04	
A (4 v. 7 minutes acid treatment)	1	5,568.52	** 58.05
B (4+7 minutes v. (twice) (0) minute acid treatment	1	55,342.56	** 576.96
C (KNO_3 v. H_2O)	1	485.68	* 5.06
D ($temperature_1$ v. $temperature_2$)	1	153.12	1.60
Treatment interactions	7	73.02	
Error (sample × treatment)	22	95.92	
Between duplicates	36	24.93	
Total	71		

ANALYSIS OF VARIANCE FOR 4- AND 7-MINUTE ACID TREATMENTS

Between samples	2	146.49	
A (4 v. 7 minutes acid treatment)	1	5,568.52	** 41.45
C′ (KNO_3 v. H_2O)	1	609.19	4.53
D′ ($temperature_1$ v. $temperature_2$)	1	221.02	1.64
Treatment interactions	4	77.19	
Error (sample × treatment)	14	134.35	
Between duplicates	24	36.39	
Total	47		

COMPARISON OF PRECHILLING AND ACID TREATMENTS

In a test begun November 29, 1938, the effect of prechilling the seed at 3° C. for 28 days was determined in comparison with that of a 4-minute sulfuric acid treatment. The pretreated samples were germinated at room temperature to 35° alternation with potassium nitrate and with tap water. The results of the germination tests are given in table 9. The acid pretreatment is better than the prechilling treatment. Only one prechilled sample showed more benefit from the use of potassium nitrate than water.

TABLE 9.—*Germination in 56 days of seed of Sporobolus flexuosus at room temperature to 35° C. alternation after prechilling or treatment with sulfuric acid Nov. 29, 1938*

Sample No.	Observations	Germination with potassium nitrate after indicated treatment			Germination with water after indicated treatment		
		Prechilled 28 days at 3° C.	71-percent H_2SO_4 for 4 minutes	Mean	Prechilled 28 days at 3° C.	71-percent H_2SO_4 for 4 minutes	Mean
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
767711	2	45.5	63.0	54.25	25.0	52.5	38.75
767741	2	17.0	59.5	38.25	20.5	51.5	36.00
767280	2	21.5	95.5	58.50	18.0	94.5	56.25
727283	2	32.0	88.0	60.00	36.5	82.0	59.25
Total or mean	8	29.00	76.50	52.75	25.0	70.12	47.56

Treatment of substratum	Total observations	Mean germination for all samples at indicated treatment		Mean
		Germination after prechilling 28 days at 3° C.	Germination after treatment with 71-percent H_2SO_4 for 4 minutes	
	Number	*Percent*	*Percent*	*Percent*
Potassium nitrate and water	16	27.00	73.31	50.15

SPOROBOLUS CONTRACTUS

GERMINATION OF INTACT SEEDS

The effect of constant temperatures of 10°, 15°, 20°, 25°, and 35° C. and of temperature alternations of 15° to 25°, 20° to 30° with light, 10° to 35°, 15° to 35°, 20° to 35°, room temperature to 35°, 20° to 40°, 25° to 40°, and 35° to 10° was determined on the germination of fresh and older seed of *Sporobolus contractus* in February and March 1938. Although all the results were low (0 to 45 percent), they indicated that the higher constant temperatures were better than the lower constant temperatures and that the temperature alternations that included higher temperatures were better than the lower temperature alternations. The use of potassium nitrate was of some benefit at the more favorable temperatures. These results are not given in tabular form.

TREATMENT WITH SULFURIC ACID

On November 28, 1938, untreated seed of *Sporobolus contractus* and seed pretreated with approximately 71-percent sulfuric acid for 4 minutes were germinated at room temperature to 35° C. alternation and at 15° to 35°. The effect of potassium nitrate was also included in this study. The results of the germination tests are given in table 10. The results with the 4-minute acid treatment are decidedly better than with no treatment. The 15° to 35° alternation was superior to that of room temperature to 35° for the untreated seed of sample No. 767287, but for the seed treated 4 minutes the difference in tempera-

ture response was not significant. The untreated and treated seeds of sample No. 767287 were benefited by the use of potassium nitrate at both temperatures. The untreated seed of sample No. 767742 germinated very little at either temperature with water or with potassium nitrate.

TABLE 10.—*Germination in 35 days of seed of Sporobolus contractus at two germination temperature alternations, with and without treatment with approximately 71-percent sulfuric acid, Nov. 28, 1938*

Treatment of substratum and sample No.	Observations	Germination after indicated periods of acid treatment at—					
		Room temperature to 35° C.			15° to 35° C.		
		0 minute	4 minutes	Mean	0 minute	4 minutes	Mean
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
767287	2	46.5	93.0	69.75	69.5	88.5	79.0
767742	2	9.5	73.5	41.5	9.5	67.5	38.5
Total or mean	4	28.0	83.25	55.62	39.50	78.00	58.75
Water:							
767287	2	20.0	81.0	50.5	39.0	82.5	60.75
767742	2	7.5	48.5	28.0	10.5	55.0	32.75
Total or mean	4	13.75	64.75	39.25	24.75	68.75	46.75
Total or means	8	20.87	74.00	47.43	32.12	73.37	52.75

Treatment of substratum	Total observations	Mean germination for the 2 temperature alternations after acid treament for—		
		0 minutes	4 minutes	Mean
	Number	*Percent*	*Percent*	*Percent*
Potassium nitrate	8	33.75	80.62	57.18
Water	8	19.25	66.75	43.00
Potassium nitrate and water	16	26.50	73.68	50.09

On February 27, 1939, untreated seed of *Sporobolus contractus* and seed treated for 2 and 4 minutes with approximately 71-percent sulfuric acid were germinated in the same manner as described above. The results of the germination tests are given in table 11. The untreated seed gave significantly lower germination than the seed treated for 2 and 4 minutes. The difference between the 2- and 4-minute treatments was not significant. The results with potassium nitrate were not significantly better than the results with water. The significant differences among the samples cannot be attributed entirely to differences in age. Sample No. 767287, which was 20 months old, had afterripened some between the two tests. On the other hand, sample No. A8763, 1-month-old seed, showed a higher germination than the 12-month-old seed of sample No. 767742. All three samples required acid treatment before maximum germination could be obtained.

TABLE 11.—*Germination in 56 days of seed of Sporobolus contractus at room temperature to 35° C. alternation after treatment with approximately 71-percent sulfuric acid for various periods, Feb. 27, 1939*

ORIGINAL DATA

Treatment of substratum and sample No.	Observations	Germination after acid treatment for—			Total observations	Mean germination
		0 minute	2 minutes	4 minutes		
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
767287	2	74.0	90.0	85.5	6	83.16
767742	2	11.0	68.5	73.5	6	51.00
A8763	2	51.5	97.0	85.0	6	77.83
Total or mean	6	45.50	85.16	81.33	18	70.66
Water:						
767287	2	51.5	84.5	61.5	6	65.83
767742	2	6.0	53.5	66.5	6	42.00
A8763	2	38.5	95.0	72.5	6	68.66
Total or mean	6	32.00	77.66	66.83	18	58.83
Potassium nitrate and water, mean	12	38.75	81.41	74.08	36	64.74

ANALYSIS OF VARIANCE FOR ALL TREATMENTS

Source	D. F.	Mean square	*F*
Between samples	2	3,002.25	
A (2 minutes v. 4 minutes acid treatment)	1	322.67	
B (2+4 minutes v. twice (0) minute acid treatment)	1	12,168.00	**36.65
C (KNO_3 v. H_2O)	1	1,260.25	3.80
Treatment interactions	2	43.0	
Error (sample × treatment)	10	331.98	
Between duplicates	18	22.75	
Total	35		

ANALYSIS OF VARIANCE FOR 2- AND 4-MINUTE ACID TREATMENTS

Between samples	2	998.37	
A (2 v. 4 minutes acid treatment)	1	322.67	1.84
C′ (KNO_3 v. H_2O)	1	726.00	4.13
Treatment interaction	1	73.50	
Error (sample × treatments)	6	175.60	
Between duplicates	12	12.50	
Total	23		

COMPARISON OF PRECHILLING AND ACID TREATMENTS

In a test begun November 28, 1938, the effect of prechilling at 3° C. was determined on the seed of *Sporobolus contractus*. The results of the prechilling tests and, for comparison, the results with seed pretreated with approximately 71-percent sulfuric acid for 4 minutes are given in table 12. The difference between the two treatments is not significant for sample No. 767287, but for sample No. 767742 the acid pretreatment is superior to the prechilling treatment. The test with potassium nitrate is significantly better than that with water except that germination of sample No. 767287 was practically complete either with potassium nitrate or with water when the seed had been prechilled.

TABLE 12.—*Germination in 56 days of seed of Sporobolus contractus at room temperature to 35° C. alternation after prechilling or treatment with approximately 71-percent sulfuric acid, Nov. 28, 1938*

Treatment of substratum and sample No.	Observations for each treatment	Germination after indicated treatment		Total observations	Mean germination
		Prechilled 28 days at 3° C.	Treated with approximately 71-percent H_2SO_4 for 4 minutes		
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Number*	*Percent*
767287	2	91.5	93.0	4	92.25
767742	2	53.5	73.5	4	63.50
Total or mean	4	72.50	83.25	8	77.87
Water:					
767287	2	94.0	81.0	4	87.50
767742	2	23.0	48.5	4	35.75
Total or mean	4	58.50	64.75	8	61.62
Total or mean for both treatments	8	65.50	74.00	16	69.75

SPOROBOLUS GIGANTEUS

GERMINATION OF INTACT SEEDS

The effect of the constant temperatures of 20°, 25°, 30°, and 35° C., of the temperature alternations of 15° to 25°, 20° to 30° with light, 10° to 35°, 20° to 35°, room temperature to 35°, 20° to 40°, 25° to 40°, and 35° to 10°, and of prechilling at 3° for various periods was determined on the germination of fresh and old seed of *Sporobolus giganteus*. The germination in any individual constant- or alternating-temperature test was less than 20 percent. The use of potassium nitrate and of light appeared to have no effect. The percentage of germination could be raised by prechilling for 28 to 56 days, but maximum germination was not obtained. These results are not given in tabular form.

TREATMENT WITH SULFURIC ACID

Seed of *Sporobolus giganteus* was treated with approximately 71-percent sulfuric acid for 5, 7, and 9 minutes and germinated along with untreated seed at room temperature to 35° C. alternation with the use of potassium nitrate and with water. The germination results are given in table 13. The tests were continued for 49 days, but there was no additional germination after the twenty-first day. The treatment for 9 minutes was superior to the other treatments for sample No. 767281, whereas the results with sample No. A8392 showed the 5- and 7-minute treatments to be superior. If the means of the two samples are considered, germination of the seed treated 7 and 9 minutes is better than that of the untreated seed or the seed treated 5 minutes. The difference between the test with potassium nitrate and that with water was significant for the different lengths of acid treatment for sample No. 767281, but was not significant for the other sample.

TABLE 13.—*Germination in 21 days of seed of Sporobolus giganteus at room temperature to 35° C. alternation after treatment with approximately 71-percent sulfuric acid for various periods, February 1939*

Treatment of substratum and sample No.	Observations for each treatment	Germination after treatment for—				Total observations	Mean germination
		0 minute	5 minutes	7 minutes	9 minutes		
Potassium nitrate:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
767281	2	4.5	45.0	60.0	72.5	8	45.50
A8392	2	19.5	71.0	76.5	61.5	8	57.12
Total or mean	4	12.00	58.00	68.25	67.00	16	51.31
Water:							
767281	2	1.0	16.0	43.5	49.5	8	27.50
A8392	2	19.0	73.5	73.5	59.0	8	56.25
Total or mean	4	10.00	44.75	58.50	54.25	16	41.87
Total or mean for both treatments	8	11.0	51.37	63.37	60.62	32	46.59

CHANGE WITH AGE

Sample No. 767281, tested in February 1938 and again in February 1939, showed no afterripening.

GERMINATION OF INTACT SEEDS OF SPOROBOLUS AIROIDES

The results of germination tests under different conditions with four samples of seed of *Sporobolus airoides* are given in table 14. The two older samples, Nos. 272724 and A8501, germinated readily and equally well at the high alternating temperatures without prechilling. Of the two samples having comparatively fresh seed, one was benefited by prechilling and the other was benefited slightly by the use of potassium nitrate. Although a high percentage of germination was not obtained with sample No. 767700, the results with the prechilled seed apparently represented the full value of the sample, for the seed germinated promptly or decayed.

TABLE 14.—*Germination in 28 to 35 days of seed of Sporobolus airoides at two temperature alternations after various treatments*

Sample No.	Date of germination test	Temperature of germination chamber (°C.)	Seed prechilled at 3° C.	Germination on substratum moistened with—	
				Potassium nitrate	Water
			Days	*Percent*	*Percent*
272724	May 18, 1938	Room to 35	0	89	94
		do	7	89	87
		do	14	95	91
		do	21	92	93
767700	Mar. 8, 1938	do	0	37	30
		20 to 35	0	43	43
		do	14	64	68
		do	21	67	66
		do	28	57	63
767716	Mar. 29, 1938	Room to 25	0	97	81
		do	14	86	84
		do	28	83	87
A8501	Feb. 28, 1939	do	0	95	95
		20 to 35	0	94	95

GERMINATION OF INTACT SEEDS OF SPOROBOLUS WRIGHTII

The seed of *Sporobolus wrightii* was germinated at four temperatures with potassium nitrate and with water and with various prechilling treatments. The results are given in table 15. The seed germinated readily at a warm alternating temperature; it did not appear to be nitrate-sensitive at a favorable temperature. Sample No. A666 appeared to be somewhat light-sensitive.

TABLE 15.—*Germination in 35 days of seed of Sporobolus wrightii at various temperatures*

Sample No.	Date of germination test	Approximate age of seed	Germination temperature alternation (°C.)	Prechilled at 3° C.	Germination on substratum moistened with—	
					Potassium nitrate	Water
		Months		*Days*	*Percent*	*Percent*
769224	Sept. 13, 1938	10	20 to 30	0	81	49
			20 to 35	0	96	87
			Room to 35	0	91	91
			20 to 40	0	88	90
			20 to 35	14	92	95
			Room to 35	14	99	93
			20 to 35	28	94	95
			Room to 35	28	97	95
A666	Feb. 28, 1939	6	20 to 35	0	76	65
			Room to 35	0	86	80

GERMINATION OF INTACT SEEDS OF SPOROBOLUS ASPER

Seed of *Sporobolus asper* was germinated at three temperature alternations with potassium nitrate and with water and with prechilling treatment. The results of the tests are given in table 16. Comparatively fresh seed appeared to require prechilling for 14 days and the use of potassium nitrate for maximum germination. Seven months later the need of prechilling and nitrate had disappeared as the seed germinated readily with tap water at room temperature to 35° C. alternation, at 20° to 35°, and at 15° to 35°.

TABLE 16.—*Germination in 49 days of seed of Sporobolus asper (sample 272725, from Woodward, Okla., 1937) at different temperature alternations*

Date of germination test and treatment of substratum	Germination at indicated temperature alternations after prechilling at 3° C. for various periods				
	Room to 35° C.			20° to 35° C.	15° to 35° C.
	0 days	14 days	35 days	0 days	0 days
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
April 16, 1938:					
Potassium nitrate	39	94	88	68	----------
Water	17	71	70	54	----------
Nov. 28, 1938:					
Potassium nitrate	96	93	----------	94	96
Water	90	96	----------	93	96

WATER ABSORPTION BY SEED OF SPOROBOLUS

Water-absorption tests were made to determine whether the effect of the coats in restricting germination of seed of *Sporobolus* spp. was

due to limiting of water absorption, as has been suggested by Jackson (*23*) and by Wilson (*38*).

The pericarp of all species studied became gelatinous in contact with water and, with the exception of *Sporobolus airoides* and *S. wrightii*, appeared to split and so release the seed. With *S. airoides* and *S. wrightii* the pericarp became gelatinous in contact with water but remained around the seed. Because the pericarp did not seem to be concerned in the coat restriction of germination, it was removed from the untreated seeds to make them comparable with the seeds from which the pericarp had been removed by acid treatment. The seeds were placed on moist blotting paper, and the pericarp was removed, under a lens, from 200 seeds of one or more samples of each species. Preliminary tests indicated that no appreciable amount of water was absorbed by the seed during removal of the pericarp. The acid-treated seeds and the nontreated seeds with pericarp removed were weighed, soaked in water for the periods indicated in table 17, and reweighed.

TABLE 17.—*Water absorption by seed of Sporobolus species*

Species	Resistance and treatment	Samples averaged	Average weight of 200 seeds				After 48 hours' soaking	Increase
			Dry	Without pericarp	After 24 hours' soaking	Increase		
		Number	*Gram*	*Gram*	*Gram*	*Percent*	*Gram*	*Percent*
S. cryptandrus	Resistant	5	0.0182	0.0170	0.0208	22.29	0.0222	[1] 25.92
	Nonresistant	8	.0171	.0160	.0195	22.23		
	Acid-treated	5		.0174	.0219	[1] 26.11		
S. flexuosus	Resistant (a)	3	.0220	.0193	.0242	25.00	.0274	[1] 42.20
	Resistant (b)	2	.0243	.0212	.0258	22.19	.0275	[1] 30.02
	Acid-treated (a)	3		.0194	.0262	[1] 34.34		
	Acid-treated (b)	2		.0215	.0279	[1] 29.57		
S. contractus	Resistant	4	.0268	.0238	.0296	24.20	.0313	[1] 36.83
	Acid-treated [2]	4		.0246	.0314	[1] 27.54	.0346	[1] 40.96
S. giganteus	Resistant	1	.0535	.0478	.0587	22.80	.0635	[1] 32.84
	Acid-treated	1		.0464	.0621	[1] 33.83		
S. airoides	Nonresistant	4	.0468	.0426	.0602	[1] 42.45		
S. wrightii	Nonresistant	2	.0492	.0440	.0610	[1] 37.70		
S. asper	Nonresistant	1	.1542	.1437	.1787	[1] 24.35	.1877	[1] 30.62
	Acid-treated	1		.1475	.1909	[1] 29.42	.1972	[1] 33.69

[1] Few to many seed showing evidence of germination.
[2] Average increase in weight after 7½ hours' soaking, 24.17 percent.

The very first evidence of germination is indicated in table 17, since it introduced another factor that greatly increased water absorption. The seed coats of none of the species studied excluded water. In *Sporobolus cryptandrus* the untreated seed of resistant and of nonresistant samples absorbed practically the same amount of water and at approximately the same rate. In *S. flexuosus* the samples labeled "resistant *a*" germinated better after acid treatment than those samples indicated as "resistant *b*"; however, there was little difference in the rate of water absorption of the two groups. The acid-treated seeds of *S. contractus* and of *S. giganteus* probably absorb water at a faster rate than the nontreated seeds. The nonresistant seeds of *S. airoides* and *S. wrightii* appear to absorb water at a faster rate than the other species. However, an error is introduced by the early germination of the acid-treated and nonresistant lots.

Although there was an indication of some increase in rate of water absorption by acid-treated and by nonresistant samples, it would not appear from these results that restriction of water absorption by the coats of *Sporobolus* seed was a cause of resistance to germination.

DISCUSSION

Much has been published on the need of light, nitrate, and special temperatures for the germination of seeds. Of the seven species of *Sporobolus* studied, *Sporobolus airoides*, *S. wrightii*, *S. asper*, and samples of *S. cryptandrus* from Colorado, Oklahoma, Washington, Utah, and Kansas germinated readily at a suitable temperature alternation, although in some cases the addition of light and potassium nitrate was necessary for maximum germination. On the other hand, *S. flexuosus*, *S. contractus*, *S. giganteus*, and samples of *S. cryptandrus* from Texas, Arizona, Nebraska, and Wyoming gave very low germination at a wide range of constant and alternating temperatures, and nitrate and light had little or no effect. Further treatment was necessary for best germination. According to Hitchcock (*21*), *S. flexuosus*, *S. contractus*, *S. giganteus*, and *S. cryptandrus* are closely related.

Harrington (*19*) and others have published on the effect of the alternation of temperatures on germination; and Crocker (*8*), Gassner (*15*), Shull (*29*), and Harrington (*19*) have pointed out the beneficial effect of high germination temperatures on intact seeds restricted in their germination by the seed coat or glumes. *Sporobolus cryptandrus* seed germinated best at a high alternating temperature and there was an indication that *S. contractus* was benefited by high temperatures, although the results with untreated seed were poor at all temperatures. In the absence of special treatment, *S. flexuosus* and *S. giganteus* germinated poorly at both high and low temperatures.

The seed of resistant samples germinated readily after treatment with approximately 71-percent sulfuric acid when supplied with the optimum conditions for germination. Concentrated sulfuric acid has long been used for overcoming the impermeability to water of hard-seeded legumes, but Hiltner (*20*), in 1910, was the first to apply sulfuric acid treatment to grass seed. Since then several workers have used concentrated sulfuric acid successfully on grass seed: Harrington (*17*, *18*) on Johnson grass and wheat, Morinaga (*26*) and Byran (*6*) on *Cynodon dactylon*, Burton (*7*) on *Paspalum notatum* and *P. dilatatum*, and Huntamer [4] and Stoddart and Wilkinson (*32*) on *Oryzopsis hymenoides*. Burton (*7*) found crude sulfuric acid of approximately 75-percent strength to be very effective. The writer (*34*, *35*, *36*), has found approximately 71-percent sulfuric acid very effective in overcoming the coat resistance in seed of *Danthonia spicata* and of *Oryzopsis hymenoides* and partially effective for *Eragrostis brizantha*. Since the resistance to germination is overcome by treatment with acid, it may be assumed that delayed germination in *Sporobolus flexuosus*, *S. contractus*, *S. giganteus*, and some samples of *S. cryptandrus* is due to some influence of the seed coat. The gelatinous pericarp apparently is not related to resistance to germination, as the species that germinated readily included both those

[4] HUNTAMER, MAY Z. DORMANCY AND DELAYED GERMINATION OF ORYZOPSIS HYMENOIDES. State College of Washington. [Unpublished thesis.]

in which the seed is released from the gelatinous pericarp and those in which the seed is retained within the pericarp.

Davis and Rose (*12*) were the first to point out the beneficial effect of stratification at 5° C. for overcoming the deep-seated embryo dormancy of *Crataegus*. Toole (*33*) and Whitcomb (*37*) used a short prechilling treatment at 5° to overcome the dormancy of freshly harvested wheat, which is due to the restriction of the coats as has been pointed out by Harrington (*18*). For the resistant *Sporobolus* species, prechilling at 3° for 2 to 4 weeks brought about maximum germination with some samples, but with most samples treatment with sulfuric acid was superior. The nonresistant samples were benefited by prechilling only when the seed was fresh.

Gassner (*15*), Magnus (*24*), and Gardner (*14*) found for various kinds of seeds that the removal of the coat or glumes eliminated the need of special germination conditions. Morinaga (*26*) found a similar condition for *Typha latifolia* and *Cynodon dactylon*, but found that breaking the seed coats of *Poa compressa* and of *Apium graveolens* did not do away with the need of alternating temperatures and other special factors. Böhmer (*5*) found that the effect of light on the light-inhibited seeds of *Nigella sativa* was the same after removal of the coat. Removal of the lemma and the palea was found by Andersen (*1*) for *Poa compressa* and by Maier (*25*) for *Phleum pratense* not to change the requirement for light for maximum germination, although Andersen found that seed of *P. compressa* would germinate without the use of potassium nitrate after the removal of the glumes. The writer found that seed of *Sporobolus cryptandrus* was still benefited by light and nitrate after modification of the coat by acid treatment or prechilling. *S. contractus* and *S. flexuosus* showed little or no effect of light or nitrate either before or after acid treatment.

The slight dormancy that may be encountered in fresh seed of *Sporobolus airoides*, *S. asper*, and nonresistant samples of *S. cryptandrus* is of short duration. The resistant samples of *S. cryptandrus* show some evidence of afterripening and there is a slight change with age in germination of seed of *S. contractus*, but even after a year or more of storage additional treatment was required for complete germination. There was no evidence that the germination response of *S. flexuosus* and *S. giganteus* changed with age of the seed.

The germination of the species of *Sporobolus* studied is rapid if all requirements for germination are supplied. Nonresistant species have completed their germination in 14 to 21 days. An occasional sample required an added 7 to 14 days for a few remaining seeds to germinate. The small amount of germination that occurs in those samples in which germination is restricted by the coats takes place quickly; the remaining seeds then remain indefinitely in a dormant condition. When these resistant samples have been treated with acid, a large portion of the germination takes place in 2 days and germination is completed in 14 to 21 days.

Crocker (*8*, *10*) has emphasized the fact that the delayed germination in seeds of many plants often may be due to the seed coats rather than to the embryos. Nobbe (*27*), Nobbe and Haenlein (*28*), Crocker (*8*), and others have reported on seeds having coats entirely impermeable to water; and Crocker (*8*, *9*) has reported on seeds having coats that limit the supply of water necessary for germination. In seed of *Alisma plantago*, Crocker and Davis (*11*) have shown that the

seed coat causes mechanical restraint. That enclosing structures interfere with oxygen absorption by the embryo and perhaps with carbon dioxide elimination as well has been shown by Crocker (*8*), Shull (*29, 30*), Becker (*3*), Gassner (*15*), and Atwood (*2*). Contrary to the belief of Jackson (*23*) and of Wilson (*38*), the seed coats of *Sporobolus* do not prevent water absorption, and a comparison of the rate of absorption by the seed of nonresistant with that by the seed of resistant samples or a comparison of nontreated and treated resistant samples would indicate that limitation of water supply is not the cause of delayed germination. It is believed that the coats might restrict gas exchange, but the writer has not worked on this phase of the problem.

SUMMARY AND CONCLUSIONS

A study was made of the optimum conditions for the germination of seven species of *Sporobolus*, of the variability of germinating power within the species, and of some special treatments for overcoming the resistance of some species to germination.

Germination percentage for *Sporobolus cryptandrus* was low at all constant temperatures, but high constant temperatures were more effective than the lower ones. An alternation of room temperature to 35° C. with light and potassium nitrate gave complete germination of the viable seeds of some samples. Other samples germinated best at these conditions, but maximum germination was not obtained. These resistant samples responded to pretreatment with 71-percent sulfuric acid. A 2-minute acid treatment was sufficient for most samples, but a few samples required 5- to 7-minute treatments. A 9-minute treatment was injurious in all cases. The treated seed responded best to the same conditions for germination as did the untreated seed. Prechilling for 4 to 8 weeks was beneficial and in general as effective as acid treatment. Small seeds removed by a 0.508-mm. sieve were of low viability. Resistant samples did not come from any one geographical region. There is evidence of afterripening; that is, seed of originally resistant samples tended to germinate better after aging.

Sporobolus flexuosus and *S. giganteus* germinated poorly and with little variation over a wide range of constant and alternating temperatures. *S. contractus* also germinated poorly at all temperatures, but there was an indication that higher constant temperatures and the temperature alternations including the higher temperatures were superior to the lower constant and alternating temperatures. The use of potassium nitrate and light had no effect on intact seed of *S. flexuosus* or *S. giganteus*, but for *S. contractus* there was some benefit from the use of potassium nitrate at the more favorable temperature. All three species responded to treatment with 71-percent sulfuric acid. Such treatment for 4, 2 or 4, and 7 to 9 minutes appeared to be sufficient for *S. flexuosus*, *S. contractus*, and *S. giganteus*, respectively. After acid treatment the seed of the above three species germinated well at room temperature to 35° C. alternation. Seed of *S. flexuosus* and of *S. giganteus* showed no evidence of afterripening after 3 to 12 months of storage. Seed of *S. contractus* 1 to 1½ years old varied in its afterripening response but still required acid treatment for maximum germination. Prechilling was not so good as acid treatment for *S. flexuosus*.

Fully afterripened seed of *Sporobolus airoides, S. wrightii, and S. asper* germinated promptly at alternation of 20° to 35° C. or of room temperature to 35°. There was an indication that fresh seed of *Sporobolus airoides* required either nitrate or prechilling and that *S. asper* required prechilling and nitrate for maximum germination. Seed of *S. wrightii* was not sensitive to potassium nitrate at a favorable temperature; one sample appeared to be somewhat light-sensitive.

The seed coat of the species of *Sporobolus* studied does not prevent the absorption of water.

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DIFFERENTIATION OF PHYSIOLOGIC RACES OF UROMYCES PHASEOLI TYPICA ON BEAN [1]

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INTRODUCTION

Two physiologic races of the bean rust organism (*Uromyces phaseoli typica* Arthur), one collected in southern California and the other in the vicinity of Washington, D. C., were differentiated in 1935 by Harter, Andrus, and Zaumeyer (*3*).[3] The one from California was designated form 1, and the one from the East, form 2. More recently Dundas and Scott (*1*), Parris,[4] Harter (*2*), and others have recognized the existence of additional physiologic races and have utilized this information in the production of bean varieties (*Phaseolus vulgaris* L.) resistant to rust.

Since the discovery of forms 1 and 2, the existence of many physiologic races has been proved from bean rust material collected from various parts of the country.

The different races described in this paper were differentiated on the basis of their parasitic action on seven varieties of beans used as differential hosts. (See table 1.)

GEOGRAPHIC DISTRIBUTION

Bean rust seems to be limited in its distribution to those regions where the relative humidity is at least 95 percent for 8 to 10 hours at a time when viable conidia are present. This requirement apparently is not met in most of the States west of the Rocky Mountains with the exception of isolated regions along the Pacific coast, nor in parts of the Great Plains. East of the Rocky Mountains, it may or may not occur, depending, it is believed, on whether or not sufficient humidity prevails. In the more humid parts of the United States, such as in many of the Southern, Eastern, and New England States, rust occurs in varying degrees almost every year. Inasmuch as many varieties are commercially resistant to several of the physiologic races, those that are grown in certain localities often determine, in part at least, the occurrence of rust infection.

The extent of infection, and consequently the amount of loss that occurs locally, depends on several factors, such as the proper atmospheric moisture and suitable temperature concomitant with the presence of viable conidia. That such a combination of conditions does not always occur is evidenced by the fact that rust may not appear more than once or twice during a period of 10 years. The lack of suitable atmospheric humidity is probably the most important governing factor where rusts

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[2] The writers take pleasure in making acknowledgment to C. F. Andrus for valuable assistance in the early stages of these investigations and to many pathologists throughout the United States and Hawaii who so kindly contributed collections of bean rust material.

[3] Italic numbers in parentheses refer to Literature Cited, p. 731.

[4] PARRIS, G. K. THE REACTIONS OF INTRODUCED BEAN VARIETIES TO RUST (UROMYCES PHASEOLI TYPICA) IN HAWAII. U. S. Dept. Agr. Bur. Plant Indus., Plant Dis. Rptr. 22: 424-428. 1938. [Mimeographed.]

occur only occasionally. Rust spores are probably present every year. Stakman et al. (*6*) have shown that the spores of wheat rust are carried long distances by the wind; this is probably true also of the spores of bean rust.

Townsend (*8*) stated that teliospores of the bean rust were not produced in Florida and that under a variety of temperature and moisture conditions most of the urediospores had lost their viability after 14 days and none were viable after 3 months in storage, a length of time not sufficient to carry the rust from the spring to the fall or winter crop in Florida. Parris [5] also stated that the urediospore was the only type found in the Hawaiian Islands. Townsend (*8*) attributes the frequent occurrence of the rust in Florida to the transport of the spores by wind currents from infected plants in regions to the north. These facts would suggest that, if suitable environmental conditions prevail, a rust epidemic might appear in any given region though no rust had occurred there for many years.

MATERIALS AND METHODS

Bean rust was collected at various places in the United States and Hawaii by the writers, or sent to them by workers in the various colleges and experiment stations. Since the rust does not develop normally in hot weather in a greenhouse, any rust material that was received in June, July, or August was placed in cold storage and held until the weather was cool enough to justify making inoculations. If the quantity of material was sufficient and the weather conditions proved favorable for infection, the differential varieties were inoculated. If the amount of spore material was insufficient for the inoculation of an entire set of differentials, it was increased by the inoculation of a few plants of a susceptible variety, usually the Pinto, from which spores were taken later to inoculate a set of differential varieties. An attempt was made to keep spore material of each physiologic race available from the time it was first collected. This could be accomplished (1) by inoculating susceptible plants at frequent intervals during the year or (2) by the storage of infected leaves. The urediospores do not retain their viability for a very long period of time, and it frequently happened that they had lost their viability or survived in so limited a number that they had to be increased before large-scale inoculations could be attempted. Poor results were obtained from inoculations made during the summer under greenhouse conditions, and outdoor inoculations increased the danger of mixing the different physiologic races.

An ordinary electric refrigerator partly solved the problem of prolonging the life of the rust spores, but even under such conditions of storage the percentage of survivals was usually small at the end of 3 or 4 months. Some of the fatalities were due to molds that parasitized the spores even in a refrigerator and destroyed their viability. Drying the leaves from 2 to 4 days in the laboratory at room temperature between layers of some absorbent paper partially eliminated the danger from molds. Some experiments on methods for lengthening the time the urediospores could be maintained in a viable condition showed that there was some correlation between temperature and longevity. From these experiments it developed that if the spores

[5] See footnote 4.

were stored in the ice compartment of a refrigerator they remained viable for many months, and good infection was obtained from urediospores stored for more than 2 years in a refrigerator cooled to −20° C.

After the adoption of the cold-storage method for maintaining viability of rust material, it was possible to have viable spores available at all times. Viability apparently was maintained for a longer period on leaves bearing the rust, although good results were obtained by storing the spores in the ice compartment of a refrigerator in small vials. While the spores stored in vials apparently did not remain viable as long as those in contact with the leaves, such storage was a convenient method and required only a small amount of space.

Pure lines of the different physiologic races were difficult to maintain. A single field collection of rust often consisted of two or three distinct physiologic races, although in practically every case one form greatly predominated, often as much as 5 to 10 or more to 1. The urediospores predominated mostly in the greenhouse, although teliospores may have developed under certain conditions on moribund leaves in about 3 weeks after inoculation. The urediospores escaped easily from the sorus and floated about in the air currents, which brought about a considerable amount of contamination. Experiments have demonstrated that urediospores will not germinate on the leaves in the open greenhouse during the winter months when artificial heat is required. On the other hand the spores remained viable for at least 15 days, and good infection was obtained when the plants were confined in an infection chamber for 24 hours. Moistening the plants before they were placed in the infection chamber was not necessary. All possible care was taken to isolate the differential varieties or other inoculated plants by placing them in different compartments and greenhouses. In spite of these precautions a certain amount of contamination resulted which sometimes confused the results. Greenhouse attendants and others engaged in watering, handling, and inspecting the plants may have transferred the spores to other inoculated or uninoculated plants. While care was taken to control insects, some were generally present which must be looked upon as agents in the dissemination of the rust spores. When the results were rendered uncertain by mixtures, the rust was pure-lined by starting with urediospores taken from a single sorus. This procedure greatly reduced the number of alien spores, but it was no guarantee of absolute purity, owing either to the presence of foreign spores on the leaf to which the spores of a single sorus were transferred or to contaminants in the sorus itself.

The experiments were conducted in the greenhouse. Conditions were not ideal but were preferable to those outdoors, where there were no barriers to the general mixing of the different races. The results from greenhouse inoculations may not be identical with those obtained under field conditions; nevertheless no considerable amount of deviation was observed, and it is believed that the data can be accepted as sufficiently dependable for all practical needs. Most of the inoculations were made between September and May. During this period environmental conditions varied greatly. In the fall and spring vigorous host plants were easily produced, but during the winter months, when there are many cloudy days, the plants sometimes lacked vigor and the rust did not develop quite as copiously and

uniformly as it did when the days were longer and there was more sunshine. The lack of uniformity of environmental conditions during the different seasons of the year accounted in part at least, for variations in amount and degree of infection of some of the differentials when duplicated at different times. These variations have been a source of confusion in interpreting the results that were obtained at different times on some of the differentials, especially those varieties that showed a certain degree of resistance.

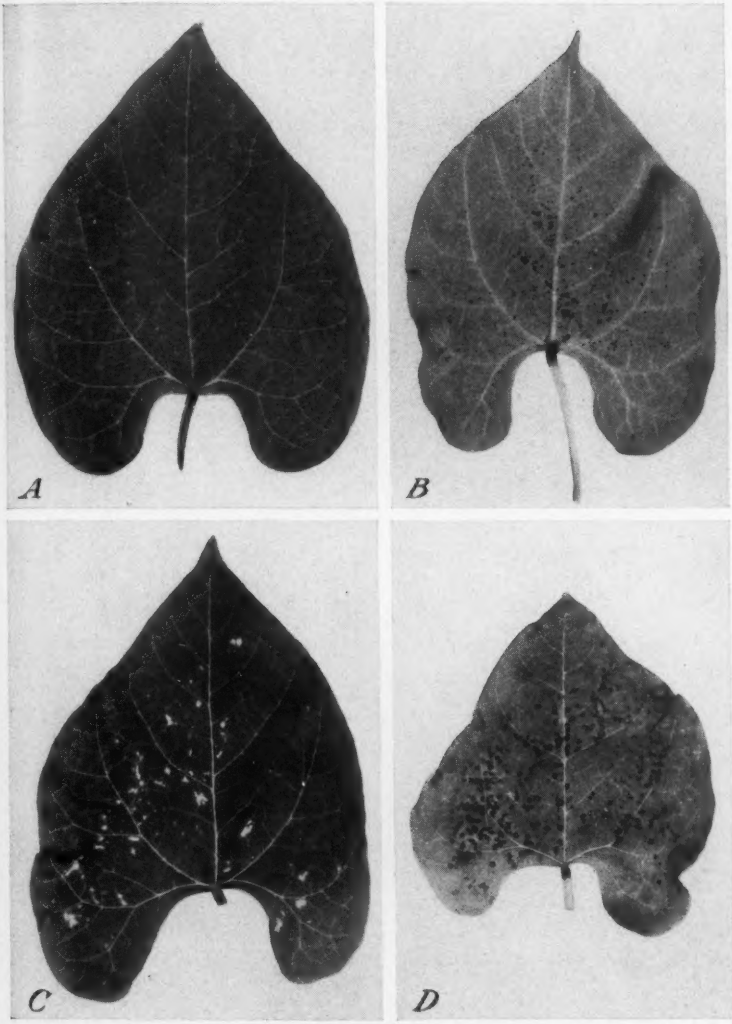
The greatest variations in results occurred mostly in the winter months, when the days were short, the light intensity reduced, and the temperature sometimes too low for the normal growth of the plants. Under such conditions, plants were often yellowed and the sori developed slowly. The percentage of infections was usually low, and mesothetic reactions occurred on certain varieties. Harter, Andrus, and Zaumeyer (*3*) found that the number of pustules on plants confined in an infection chamber in subdued light decreased as the length of time in the infection chamber beyond 48 hours increased. The decrease in the amount of infection was attributed largely to a lowering of the functional activity of the plant in the absence of sufficient light to support normal growth. Wei (*9*) stated in this connection that temperatures ranging from 16° to 28° C. did not change the type of reaction, but in some cases both the high and low temperatures increased the proportions of the X type [6] of infection. Low temperatures increased the incubation period.

The seven differential varieties of beans employed in identifying the various physiologic races considered here were as follows: (1) U. S. No. 3, a white-seeded Kentucky Wonder type; (2) Bountiful (No. 181),[7] a common garden bean variety of the bush type; (3) a strain of California Small White (No. 643), a field type grown principally in California; (4) a strain of Pinto (No. 650), a speckled field bean grown extensively in the intermountain region of the West; (5) a selection (No. 765) from the Kentucky Wonder Wax variety; (6) a medium-late white-seeded Kentucky Wonder hybrid (No. 780); (7) a brown-seeded Kentucky Wonder hybrid (No. 814). Seed of each of the differential varieties originated from a single plant selection and was increased each year in Colorado. Occasionally an off-type plant appeared in some of the varieties, which doubtless resulted from an occasional natural field cross with some other variety. Off-type plants were removed so that only rarely was there any variation in the reaction of any of the plants of a single differential. The different rust collections were identified from the reaction on the seven differential hosts described above.

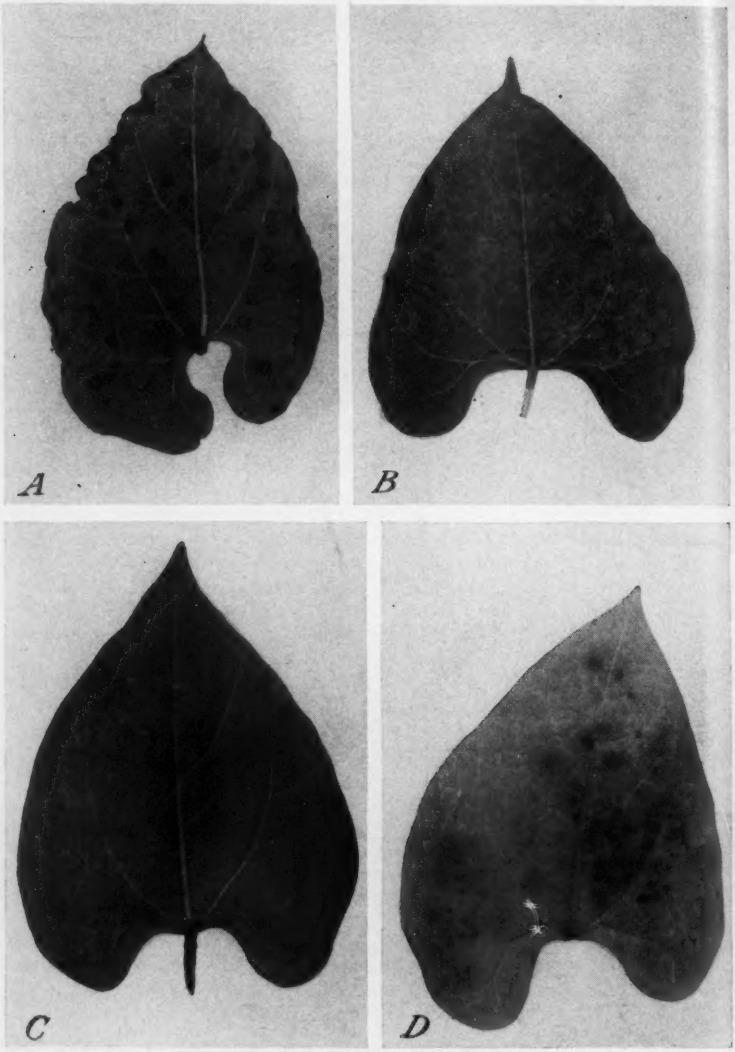
Inoculations were made by brushing spores suspended in water on the upper or lower surface of the primary leaves by means of a camel's-hair brush when the leaves were about one-half to two-thirds grown. Just as good infection was obtained by dusting the spores on the dry leaves. Since the simple leaves matured 7 to 10 days before the trifoliate leaves were fully grown, they were preferred for inoculation purposes. Identical results were obtained with both types of leaves if all other conditions were alike. Young leaves were preferred. Experiments showed that if old simple leaves were inoculated infection took place but the sorus did not attain its maximum

[6] Used as the equivalent of mesothetic.

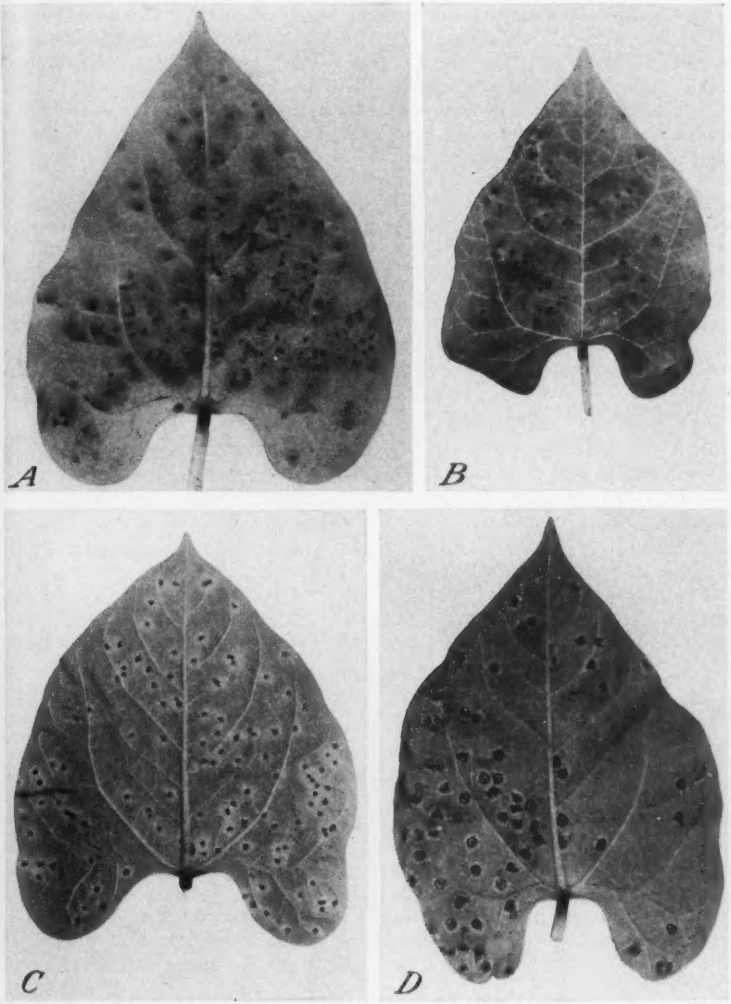
[7] The numbers in parentheses following varietal designations are pure-line selections carried under these numbers in the seed files of the writers.



Types of infection, grades 0, 1, and 2, produced by several races of rust on different varieties of beans. *A*, Grade 0 infection. Brown-seeded Kentucky Wonder hybrid (No. 814) inoculated with rust race 2, showing the highest degree of resistance. *B*, Grade 1 infection. The necrotic lesions are small and more or less round in outline, showing a high degree of resistance. No spores in the sori. Kentucky Wonder Wax (No. 765) inoculated with race 10. *C*, Grade 1 infection. Note the difference in the type of lesions from those shown in *B*, Kentucky Wonder Wax (No. 765) inoculated with race 1. *D*, Grade 2 infection. The lesions differ in type from those shown in *B* and *C*. A few spores occur in some of the sori. U. S. No. 3 inoculated with rust race 1.



Types of infection, grades 2, 3, and 4, produced by several races of rust on different varieties of beans. *A*, Grade 2 infection, showing a different type of lesion from those shown in plate 1, *B*, *C*, and *D*. Pinto variety inoculated with rust race 10. *B*, Grade 3 infection. Note difference in the type of lesions from those in *A*. White-seeded Kentucky Wonder hybrid (No. 780) inoculated with rust race 12. *C*, Grade 3 infection. A different type of lesion from those shown in *A* and B. There is very little necrotic tissue outside the sori. White-seeded Kentucky Wonder hybrid (No. 780) × Pinto inoculated with race 9. *D*, Grade 4 infection. White-seeded Kentucky Wonder hybrid (No. 780) inoculated with rust race 7.



Types of infection, grades 5, 6, 8, and 10, produced by several races of rust on different varieties of beans. *A*, Grade 5 infection. Kentucky Wonder Wax (No. 765) inoculated with race 14. *B*, Grade 6 infection. California Small White (No. 643) inoculated with race 15. *C*, Grade 8 infection. White-seeded Kentucky Wonder hybrid (No. 780) × Pinto, inoculated with race 11. *D*, Grade 10 infection. Pinto inoculated with rust race 2. Secondary rings occur in connection with many sori.

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size. If the physiological activity of the leaf was much reduced by aging before the pustules were mature, they remained undeveloped even on highly susceptible plants.

Immediately following inoculation the plants were placed in infection chambers and kept there for 24 to 48 hours, when they were removed to a bench in the greenhouse. An incubation period of 24 hours was as good as or better than any longer time. The infection chambers were constructed on one of the regular greenhouse benches. The front and back of the bench were built to the height of 1.5 and 2 feet, respectively, and the compartment was covered with a glass sash hinged at the back. Wet sphagnum was placed at the bottom of the chamber in order to maintain a high humidity. The infection chamber was divided into several individual compartments, each large enough to accommodate 100 or more pots. Under favorable greenhouse conditions, infection was evident in from 5 to 6 days after inoculation. The sori began to open a few days later, and the records of the degree of pustule development were made about 14 days after inoculation. They were sometimes taken a day or two later if it was suspected that development of the rust was delayed because of low temperatures or for other causes.

INFECTION RATING

The size of the rust pustule at the end of 14 days was the criterion for fixing the degree of susceptibility and resistance. Harter, Andrus, and Zaumeyer (*3*) previously used a scale of 0 to 10, 0 denoting immunity and 10 the highest degree of susceptibility, with intermediate grades between these two extremes. It is proposed to employ the same scale of graduation in this paper. Other investigators have used the same system, while some have used a scale of 0 to 100.

DESCRIPTION OF GRADES AND TYPES OF INFECTION

Grade 0.—Totally immune (pl. 1, *A*), no lesions or other evidence of infection.

Grade 1.—Necrotic flecks without spores (pl. 1, *B* and *C*). There is considerable variation in the general characteristics of the flecks on several of the differential varieties, caused by the various physiological races of the organism; some are very small and round, somewhat resembling the point of a needle (pl. 1, *B*), while others are angular in shape and vary greatly in size (pl. 1, *C* and *D*).

Grade 2.—This grade (pl. 1, *D*, and pl. 2, *A*) differs from grade 1 largely in that, although the sori are small, some spores are produced. The infection centers may or may not be surrounded by a necrotic area. Plants falling in this class are highly resistant.

Grades 3 to 10.—These grades (pl. 2, *B*, to pl. 3, *D*) are differentiated on the basis of the size of the spore-bearing pustules. Grade 3 (pl. 2, *B*, *C*), grade 4 (pl.2, *D*), grade 5 (pl. 3, *A*), and to a less extent grade 6 (pl. 3, *B*) are regarded as commercially resistant, and higher grades up to grade 8 (pl. 3, *C*) are regarded as possessing some degree of tolerance.

Secondary and tertiary rings of sori (pl. 3, *D*) developed in many varieties of beans under certain conditions; however, their formation was not constant enough to be of any value in race identification. Rings were produced on some varieties more readily than on others, and it was by no means the more susceptible, but frequently the moderately susceptible ones, on which they appeared first. The

Bountiful, a variety that is not highly susceptible to any of the rust forms with the possible exception of race 10, frequently exhibited secondary rings in about 10 days and often earlier. There appears to be some correlation between the size of the secondary rings and resistance. It may be stated that, in general, secondary and tertiary rings were not produced in infections below grade 6. On the other hand, they were to be expected on all varieties with infections from grades 6 to 10.

Under greenhouse conditions susceptible and resistant plants inoculated with the same physiologic race often showed very different symptoms in the initial stage. Visible signs of infection appeared in about 5 days as small, nearly round and nearly white, immature pustules on the under side of the leaf beneath the epidermis. These juvenile sori gradually enlarged and in 7 to 10 days ruptured the epidermis. On the other hand, resistant plants of grade 1 and occasionally grade 2 usually showed brown necrotic lesions on the under side of the leaf in about 3 days. The lesions increased slightly in size and became a little darker in color, but there was little if any enlargement after 5 days. It was usually possible to determine resistant plants of grade 1 or 2 before there were any indications of infection in susceptible varieties.

RESULTS

IDENTIFICATION OF PHYSIOLOGIC RACES

Twenty different physiologic races of bean rust have been identified. Each one was separated from the others by its reaction on seven differential bean varieties. In only a few cases were any of the differential varieties entirely immune. There were, however, many examples of differentials being resistant, tolerant, or fully susceptible to different races. It was therefore frequently necessary to make use of degrees of infection of the differential varieties for the separation of the different races. In most cases degrees of infection were reasonably stable, although occasionally there were some departures from the standard, which sometimes rendered identification somewhat difficult. These variations in results occurred mostly with those varieties that showed some degree of resistance or were grown under unfavorable environmental conditions. For the most part they belonged to that group of varieties which the writers have termed commercially resistant.

A comparison of the reaction of a rust on the differential varieties with the data contained in table 1 should make identifications reasonably simple. A too close agreement must not always be expected, inasmuch as environmental conditions are known to influence the degree of infection, and mesothetic types sometimes confuse the results. Several tests may be required, but usually only one or two are necessary.

Reference to table 1 will show that U. S. No. 3 and No. 765 gave a "fractional" reaction to some of the races, that is, a difference in the degree of infection on the upper and lower sides of the leaf, the two readings being expressed in the form of a fraction. This behavior was not characteristic of any of the other differential varieties except No. 780 inoculated to race 19. U. S. No. 3 has shown an inconsistent tendency to fractional reactions with other physiologic races besides 6, 7, 8, and 20.

TABLE 1.—*Reaction of the differential bean varieties to various physiologic races of bean rust (Uromyces phaseoli typica)* [1]

Differential variety	Infection grade [2] produced by physiologic race—																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
U. S. No. 3	2	10	9	2	10	5-6	7	5	10	3	10	10	10	2	10	5	10	2	10	9
						3	3	3												7
No. 181	8	8	8	8	8	8	8	9	9	9	8	8	8-9	8	8	8	7	7	8	8
No. 643	9	8	2	1	9	9	9	1	9	1	9	10	9	8	5-6	9	1	8	9	9
No. 650	10	10	10	10	10	10	10	10	10	2	10	10	10	10	10	10	10	10	10	10
No. 765	1	2	2	2	2	2	2	1	5-6	1	6	2	9	4-5	2	4	5	2	4	5
																2				2
No. 780	1-2	1	1	2	1-2	2	4	2	2-3	1	6	3	10	9	2	9	1	5	4	9
																			2	
No. 814	1-2	0	9	9	10	9	9	9	10	0	10	0	9	10	9	9	0	8	8	9

[1] These records represent the results obtained under the authors' criterion of the most favorable conditions. If environmental conditions are not favorable some variation must be expected, especially in the case of those varieties showing a high degree of resistance.

[2] Infection grades range from 0 for immunity to 10 for highest degree of susceptibility. The fractional expression of grade indicates different reactions on (numerator) upper and (denominator) lower surfaces of the leaf.

California Small White (No. 643) reacted peculiarly in several respects, and occasionally it was difficult to classify the several races as to the degree of infection. The lesions or pustules sometimes varied from grade 3 or 4 up to 8 to 10 on the same leaf when inoculated with a pure-line race. The infection was classed according to the grade that predominated on all the inoculated plants. This variety has consistently produced a small number of pustules in comparison with the other differential varieties.

Physiologic race 5 appears to be more prevalent and more widely distributed than most of the others. The investigations showed that there were a number of strains, closely related to but not identical with race 5, which did not differ enough from it to be classed as different races. The differences were mostly confined to the differential varieties Nos. 780 and 765. In table 1, race 5 is graded as 2 on No. 765 and 1 to 2 on No. 780; however, in the case of some strains of rust, a reading of 1 or perhaps 3 may occur on these two differential hosts. In other cases the grade may be below 2 on one of these differentials and a grade higher on the other. If there was no variation or only very little in the reaction of the differentials to the rust in duplicate tests, such differences might be sufficient to justify classifying the rust as a new race. In the absence of such constancy, the only other alternative seems to be to recognize the existence of subraces. In this connection it is proposed to create four subraces of race 5: Race 5–a includes those rusts that grade 0 on No. 780 and No. 765; 5–b, those that grade 1 on No. 780 and No. 765; 5–c, those that grade 1 on No. 765 and 0 on No. 780; and 5–d, those that grade 0 on No. 765 and 1 on No. 780, according to the scale adopted by the writers.

All the differentials except No. 181 showed considerable resistance to race 10. The Pinto (No. 650), a variety highly susceptible to all the other races and the one used as a standard with which reactions on all the differentials were compared, is quite resistant to race 10. It might appear that race 10 is a weak strain of rust; however, a large number of bean varieties are highly susceptible to it, as reference to table 2 will show. It was collected on the Bountiful variety, which was employed to keep the rust in an active condition in the greenhouse. While No. 780 and No. 765 are both resistant or at

least tolerant to most of the physiologic races, No. 780 is highly susceptible to races 13, 14, and 16 and No. 765 to race 13.

REACTION OF BEAN VARIETIES TO PHYSIOLOGIC RACES

The results of the relative susceptibility and resistance of a number of garden and field beans to one form of bean rust were published in 1935 (*3*). These varieties were graded on a scale of 0 to 10 and divided into three classes: Very susceptible, moderately susceptible, and slightly susceptible. The results showed considerable variation, some being highly susceptible while others were immune or highly resistant. In general the field beans were found to be more susceptible than the garden varieties.

In the present paper the varieties are graded on a scale of 0 to 10, according to the description given earlier for each grade. As new races were discovered they were inoculated to the different varieties; there now have been obtained ratings for resistance of most of the commercial varieties (table 2) to all rust forms except races 15 to 20.

TABLE 2.—*Reaction of bean varieties to 14 physiologic races of bean rust*

Group and variety	Infection grades [1] produced by physiologic race—													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Field beans:														
Blue Pod	10	10	9	5	10	10	10					10	10	10
Boston Marrow	8	7	7	7	7	7	3			2				
Brown Swedish	10	8	8	8	7	6	4		7	10	8	7	5	
Great Northern	10	10	10	10	10	10	10	10	10	10	1	10	9	8
Kotenashi	10	10	9	10	10	9	10	8	10	1	10	8		
Large White Marrow	10	10	9	10	10	8	10	10	10	0	10	10		10
Marrow, Pea	10	10	10	10	9	9	7	10	10	9	10	10	10	9
Michelite	8	10	9	10	10	8	8	10	9	3	8	9	8	6
Otenashi	10	10	9	10	10		9	10	10	0	10	9	8	10
Perry Marrow	8	5	7	7	7	7	6	3	10	1	6	10	7	8
Pink	9	10	10	10	10	10	10	10	10	1, 5				
Pinto	10	10	10	10	10	10	10	10	10	2	10	10	10	10
Red Kidney, California strain	8	6	7	5	10	8	5	7	7	10	8	5	8	8
Red Kidney, Geneva	7	7	7	7	6	1	6		7	1	8	3, 5	5	8
Red Kidney, Wells	8	8	7	7	7	6	6							
Red Mexican	10	10	10	10	10	10	10			1		10	9	10
Robust	9	9	2, 10	10	10	10	10	10	10	2	10	10	10	10
Tepary [2]	10	10	10	9	9	9	9	4	7	2, 5	9	9		10
White Kidney	3, 8	5, 8	7	8	8	8	7	4	6	3	5	3, 5	6	
White Pea-bean, Geneva	10	10	9	10	10	10								
Yellow Eye, Improved	8		1, 6					5	7	10	6	5		1
Garden beans (pole):														
Blue Lake (White Creaseback)	8	10	10	10	10	10	10	10	10	10	10	10	8	9
Caseknife, Early June	10	10	10	10	10	10	10	10	10	0	10	10	10	10
Caseknife, Dutch	6, 9	10	9	2	10	7/4	2	8	10	0	10	10	2	5/2, 9
Cutshort (Corn Hill)	10	10	10	10	10	10	9	10	10	2	10	10	10	10
Everbearing	6	7	8	7	8	1	2							
Golden Cluster Wax	4, 7	9	10	10	10	10	2, 10	10	10	9	10	10	10	10
Horticultural, King Mammoth	2	9	7	2	7	1, 3		0	1	8	4/2	0		0
Horticultural, London	1	9	8	0	7/3	1	0, 3	0	1	8	1	0	0	0
Ideal Market (Black Valentine pole)	8	10	9	10	10	9	10	10	10	8		10	9	9
Ivory Stringless	1, 0	8	8	0	7/3	1	1, 4						6	0
Kentucky Wonder (Burger Stringless)	10	10	10	10	10	10	9	10	10	2	10	9		10
Kentucky Wonder (brown-seeded)	10	10	10	10	10	10	10	10		5			8	10
Kentucky Wonder (Morse 191)		10		2	10		10					10	4/2	4/2
Kentucky Wonder, Rust Resistant	2	1					9	6	9	1	10	9		9
Kentucky Wonder, U. S. No. 4	1	10	10	2	10	8/3	3	6	10	2	10	7		10
Kentucky Wonder Wax	2, 6	4, 8	9	2	9	9/3	1	2	9	0	10	4/2	4	5/2
Kentucky Wonder (white-seeded	10	10	10	10	10	2	2	9	10	3	10	2	3	5/3
Lazy Wife (White Cranberry)	1	9	7	0	6/2	1	0	8	0	8	4/2	0	8	0
McCaslan	9	2, 10	10	8	8	10	10	10	10	2	10	10	10	10
Missouri Wonder	10	2, 10	10	10	10	2								

[1] Infection grades range from 0 for immunity to 10 for highest susceptibility. Two or more whole numbers indicate that different plants reacted differently. Fractions indicate different reactions on upper (numerator) and lower (denominator) surfaces of the leaf.

[2] *Phaseolus acutifolius latifolius* Freeman.

TABLE 2.—*Reaction of bean varieties to 14 physiologic races of bean rust*—Continued

Group and variety	Infection grades produced by physiologic race—													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Garden Beans (pole)—Continued.														
Oregon Giant	---	10	9	4	10	6/2	2	2	10	10	10	4/2	7	10
Phenomenon	2	10	10	4/2,10	10	7/3,10	2	5/2	10	1	10	10	1	10
St. Louis Perfection, White	10	10	10	10	10	10	---	---	---	---	---	---	---	---
Striped Creaseback (Scotia)	10	10	---	10	10	10	10	10	10	10	10	10	10	10
Tennessee Wonder (Brown Sickle)	10	10	10	6	10	2	10	6	10	2	10	6/3, 8	10	10
Zeppelin pole	2	10	10	4/2	10	3	2	---	10	2	9	9	---	10
Garden beans (bush):														
Asgrow, Stringless Green Pod	9	9	8	9	8	2	2	4	8	10	8	6	1	1
Asgrow, White Seeded Wax	7	9	8	8	8	2	2	---	9	3	8	7	1	1
Black Seeded Green Pod	8	7	6	8	9	---	2	---	7	10	8	7	1	1
Bountiful	8	8	8	8	8	8	8	9	9	9	8	8	8–9	8
Brittle Wax (Round Pod Kidney Wax)	9	9	6	9	10	---	2, 6	6	10	10	8	8	2	1
Burpee Stringless Green Pod	4	9	8	6	5	5	4	---	7	10	8	8	6	4
Canadian Wonder	6	8	6	6	8	5	6	3	7	4	7	7	5	6
Challenger Black Wax	10	9	8	6	8	3	3	---	8	10	8	6	6	1
Commodore	---	8	---	8	10	---	1, 3	2, 4	8	10	7	8	1	---
Currie Rust Proof Black Wax	3	8	7	7	6	2, 7	2, 4	2, 4	7	10	8	7	1	1
Davis Stringless Wax	7	8	8	7	7	6	6	6	5/7	10	8	7	7	6
Dwarf Black Wax	6	8	8	8	8	2	2, 5	4, 8	8	10	9	9	3	1
Dwarf French Master	9	9	8	8	7	7	4, 6	---	8	4	8	6	6	6
Early Mohawk	9	9	8	7	9	2	2	3, 6	8	10	8	8	1	---
Flageolet Wax	6	6	6	7	2/6, 6	1	---	---	---	---	---	---	---	---
Flageolet White	1	8	8	1	8/4	0	0	---	---	10	---	3	0	0
Fordhook Favorite	2	8	8	6	7	1	4	3	7	10	9	6	6	1
Full Measure	4	10	9	9	7	8	6	2	7	10	7	7	---	5
Giant Stringless Green Pod	3	9	9	9	8	9	---	8	8	10	9	8	---	8
Goddard (Boston Favorite)	8	7	8	8	10	9	8	7	8	10	8	9	---	8
Golden Crown	10	10	10	10	10	10	9	---	---	---	---	---	---	---
Golden Eyed Wax	8	6	6	5	9	---	2	4	7	10	10	9	6	1
Golden Sword	1	5, 9	8	0	3	1	1	0	0, 3	10	5/3	1	---	---
Grennell Rust Proof Wax	10	7	8	7	8	2	2	6	8	10	8	5	1	1
Hardy Wax	8	7	6	8	0	---	1	4	8	10	7	6	1	1
Hodson Wax	2, 4	9	10	10	9	2	2	7, 9	10	10	10	8	---	1
Horticultural, Dwarf	8	7	8	8	7	8	7	8	7	10	9	8	8	8
Horticultural, Dwarf (Cranberry)	0	7	8	0	6/3	0	---	6	8	10	8	9	8	---
Horticultural, French	2	8	9	3	7	3	2	1	8	10	8	2	---	2
Improved Black Wax	7	9	8	8	7	2	3	3, 5	8	10	8	8	2	2
Improved Golden Wax	1, 3	9	8	8	8	2	8	6	8	9	8	7	1	1
Improved Kidney Wax	3	8	8	9	7	2	2	2, 5	8	10	8	5, 7	1	---
Keeney Rustless Golden Wax	4	7	7	5	5	2	3	4	5	10	8	---	5	1
Konserva	9	10	7	8	7	7	7	8	7	10	8	6	9	8
Longfellow	5	9	8	9	6	7	6	5	8	10	8	6	7	9
Low Champion	2	7	8	8	6	0	2	3, 5	7	10	8	8	1	1
Masterpiece	10	10	10	10	9	9	10	3,4,8	9	1	7	7	7	8
Michigan White Wax	7	7	6	9	6	6	6	3, 6	7	10	9	10	---	---
New Sioux Stringless Wax	9	9	9	9	8	8	8	8	8	10	8	7	7	8
Nonpareil	10	10	10	10	10	10	10	10	10	2	10	10	9	10
Pencil Pod Black Wax	3	7	8	9	7	2	2	6	8	10	8	7	2	1
Plentiful	5	7	5	7	10	5	5	---	5	9	5	8	6	7
Prolific Black Wax	8	7	8	8	7	2	2	4, 9	5	10	8	5	2	2
Refugee, 1000–1	2	5	6	6	7	8, 2	6	---	4	10	5	7	2	1
Refugee, Corbett	8	6	10	10	8	8	8	---	---	8	---	9	9	9
Refugee, Early	2, 7	8	7	8	7	1	2	---	4	10	5	3, 5	2	1
Refugee, Keeney Stringless Green pod	8	8	8	8	6	6	2, 4	---	7	8	6	6	2, 8	1
Refugee, Idaho	10	7	7	8	9	7	7	---	9	10	6	9	9	7
Refugee, U. S. No. 1	9	9	7	7	7	7	---	---	---	---	---	8	7	8
Refugee, U. S. No. 5	7	7	7	7	7	7	7	4	4	3	6	7	---	5
Refugee Wax	3, 7	8	7	8	6	2	1	---	4, 7	10	7	4, 6	2	1
Refugee, Wisconsin	6	4	8	8	8	1	2	---	8	8	5	8	1	1
Refugee, White-Seeded	8	6	4	3	8	---	1	6	4	---	6	6	2	1
Royal Purple Wax	6	8	6	6	7	3	4	5	5	8	5	5	---	---
Rust Proof Golden Wax	2	8	7	7	8	2	2	5, 7	8	10	8	7	2	1
Stringless Kidney Wax	7	6	6	6	8	2	---	10	8	10	---	8	---	1
Superba	8	9	8	9	8	2	3/2	---	3	2	6	7	---	1
Sure Crop Wax	3	8	8	8	8	2	0	5	8	10	---	7	2	1
Tendergreen	6	9	7	7	8	2	2	3	6	10	7	8	1	6
Tennessee Green Pod	10	10	10	10	10	10	10	8	10	0	10	8, 10	10	10
Unrivaled Wax	7	9	8	8	7	7	6	3	---	10	8	9	7	9
Valentine, Asgrow Black	6, 8	7	7	7	7	7	7	4	4	3	6	7	1	1
Valentine, Black	3	9	8	5, 8	7	1	3	4	4	10	4/7	3	6	1
Valentine, Red	4, 7	7	7	8	7	8	2	5	7	10	7	6, 8	1	6
Valentine, Red Stringless	8	8	7	8	7	6	7	6	6	10	8	5	0	7
Wardwell Kidney Wax	3	8	6	7	8	2	3	5	7	10	5	7	---	0
Weber Wax	1	9	8	9	9	2	3	4	9	10	7	7	1	2

Most of the seed for the variety tests was furnished by commercial seed growers, and in nearly all cases it was reasonably uniform in type and reactions, although considerable variation was occasionally found within some varieties, and this sometimes ranged from grade 0 to 10. The lack of uniformity occasionally confused the results, although in most cases the percentage of off-type plants was small. If the data indicated mixtures the grade of rust was determined from the reaction of the majority of the plants.

In general the field beans appear to be more susceptible to most of the physiologic races than the garden varieties. A striking exception is the case of race 10, to which the garden beans are more susceptible than the field varieties. Some of the garden varieties (Blue Lake, Early June Caseknife) are highly susceptible to most of the races, and some of the field beans (Geneva Red Kidney, White Kidney) are tolerant. In both the field and garden beans there are varieties (Oregon Giant, Great Northern, Brittle Wax, Hodson Wax) that are highly susceptible to some races of rust and resistant or tolerant to others.

Among the garden beans the pole varieties appear to be more susceptible than the bush types. The Kentucky Wonder types are naturally very susceptible and are often seriously damaged in regions where rust occurs. The Blue Lake, Early June Caseknife, and Cutshort varieties possess little or no tolerance to any of the rust forms, and there are several other varieties that are susceptible to all the races with one or two exceptions. On the other hand the King Mammoth Horticultural and London Horticultural, two pole varieties, are resistant or tolerant to more races than Dwarf Horticultural, a closely related variety of the bush type.

GEOGRAPHIC DISTRIBUTION OF PHYSIOLOGIC RACES

The geographic prevalence and distribution (table 3) of the different physiologic races in the United States were determined from rust material collected wherever found by the writers and from similar material sent them by pathologists and others from some of the agricultural colleges and experiment stations.

Previous to 1927, bean rust, although known to occur in many parts of the United States, was not as generally prevalent or destructive as it has been since. Sporadic outbreaks were reported by various investigators or observed by the writers in localities where very susceptible varieties were grown and the environmental conditions were favorable for rust infection; but epidemics such as some of those in recent years were exceptional, if they actually occurred.

After the rust had reached the epidemic stage over wide areas in several localities, a search was instituted for resistant varieties, and this eventually led to the discovery that not only one but possibly several different physiologic races occurred. The number of such races and their geographic distribution, as well as the reaction of different varieties to different races, were facts of much importance if attempts were to be made to develop resistant varieties by hybridization and selection. Preliminary inoculation tests with rusts collected from various parts of the country showed considerable diversity in results. Some varieties were highly resistant to a rust collection from a certain source but highly susceptible to one collected in a

different region. This information has been utilized in selecting material for the development of rust-resistant progenies.

The data shown in table 3 are too meager to be highly significant although some facts of interest are evident. The most outstanding lesson to be drawn from the results of the accumulated data is that there are no races specific for or representative of any one locality. The evidence shows that the races established in a locality in any one year may be followed by a different race or group of races the next year. In 1936 races 1 and 2 were identified from material obtained from Florida and some of the other States. In 1937 few collections were identified, but in 1938 race 1 was received only three times, and race 2 has been collected but once since 1936, and then from California. In 1938 races 5, 9, 10, and 11, and in 1939 race 5 were identified from Florida, but races 1 and 2 have not been received from that State since 1936. Races 1, 2, 3, and 4 were collected in Virginia in 1936; races 10, 14, and 16 in 1938; and race 10 in 1939. The few examples cited are typical of the entire country so far as physiologic races of bean rust are concerned.

TABLE 3.—*Distribution of the different physiologic races of bean rust collected in the United States and Hawaii, 1936–39*

State or Territory	Distribution of physiologic races of bean rust by years			
	1936	1937	1938	1939
Arkansas			5	
California			1	1, 2, 5, 10, 13
Colorado	1	4, 7, 8	1, 9, 13	
Florida	1, 2		5, 9, 10, 11	5
Hawaii			1, 12	
Louisiana			12	
Maine	4		5, 17	
Maryland	5		10	10, 20
Massachusetts	1, 2		5	
Michigan				5, 18
Nebraska			5	
New Jersey	4, 5		10	
New York			3, 10	
North Carolina			5	19
South Carolina	1, 2		15	
Virginia	1, 2, 3, 4, 6		10, 14, 16	10
Washington		1, 4		18
Wisconsin			5	

DISCUSSION

The results of the investigations justify certain inescapable conclusions. It became apparent early in the prosecution of the problem and was more clearly demonstrated later that it was not always possible to obtain identical results from duplicate experiments. Differential host varieties often gave a slight difference in the degree of rust infection when inoculated with the same physiologic race at different times. In view of this fact it is essential that a too close agreement with previous results must not be expected. These variations were due to several causes. The greatest variations were obtained between inoculations made in the winter months and those made in the late spring and early fall, when the days were longer and the amount of sunshine much greater. Beans appear to thrive best with the maximum amount of sunshine and fairly high temperatures. During the winter months in the vicinity of Washington,

D. C., there are many cloudy days and frequently many consecutive days without sunshine. Under such conditions the plants lack vigor and are often icteroid. There seemed to be a direct correlation between plant vigor and the size of the sori. The sori on plants grown under reduced light were generally pale and smaller than those on plants grown with the optimum amount of sunshine. Temperature likewise influenced the size and general appearance of the sori and the tissue immediately surrounding them. If the plants were held in incubators or in an environment where the temperature was too high, the leaves became pale and the sori were undersized and slow to develop.

These inconsistencies and variations in the behavior of rust forms, attributed partly to environment, may possibly be explained in accordance with observations made by cereal rust pathologists who have proved the occurrence of mutations of various sorts in wheat rust. Newton and Johnson (*5*) selected a bright-orange and a grayish-brown mutant out of *Puccinia graminis tritici* (Pers.) Eriks. and Henn. Neither of the mutants showed any change in infection capability on the differential hosts. Both, however, differed markedly in viability and in the length of their urediospores. Johnston (*4*) secured from wheat in Texas a race of rust that differed in several ways from other known races. He found that its incubation period and time for full development were much longer than for other forms. The spores were lighter in color and smaller in size. He suspected, but offered no proof, that it may have originated as a mutation.

Interesting in this connection are the results of Newton and Johnson (*5*), who found mutations of wheat rust to be induced by the refrigeration of the urediospores. The mutation occurred in a form of rust that had been constant for 2 years. After the urediospores had been in storage for 6 months at a temperature of 8° C., inoculation experiments indicated a mixture of the original and a new form of rust. Four months later a new race had completely supplanted the old race and gave an entirely different reading on the differential hosts.

These facts may explain some of the difficulties the writers have experienced. It has been the practice to collect urediospores and store them in a refrigerator at temperatures below 0° C. for various lengths of time, or until they were needed. Viable spores have been kept in storage for more than 2 years, and markedly different results have been observed when they were used to inoculate plants at a later date. In no case, however, has a complete change been observed or has any new race been developed. The results, a priori, suggested a contamination but under conditions that made it difficult to explain how it came about. What happened to the race of wheat rust described by Newton and Johnson (*5*) may have occurred in bean rust that was stored by the writers at a considerably lower temperature.

More significant still are the results of Stakman, Levine, and Cotter (*7*), who demonstrated that not only did mutation take place in cereal rusts but that new races were isolated as the result of mixing the nectar of such species as *Puccinia graminis tritici* and *P. graminis agrostidis* Eriks. These results indicate that new physiologic races may originate from several causes.

The sori on the leaves of highly susceptible plants grown under optimum conditions were uniform in size and produced an abundance

of urediospores over a considerable length of time. On the other hand, not only may partially resistant or tolerant plants have a comparatively small number of sori but there may be several grades of infection, as for example grades 3, 7, 10, on the same leaf. So far as bean rust is concerned, a variety may react mesothetically to one or more physiologic races and not to others. California Small White (No. 643) frequently showed such a range in the grade of the sori that it was sometimes difficult to determine to which class they belonged. While it was highly susceptible to some physiologic races, there may have been many undeveloped sori that might fall in any grade from 3 to 10.

Differences in reaction among several stocks of a single variety have been the cause of considerable confusion. The Refugee is a good example. This variety is widely grown commercially. There are several different strains all of which doubtless originated from the same parental stock. However, the different seed companies have selected lines according to their own fancy and have obtained strains that react quite differently to a single physiologic race of rust.

How have new races of bean rust originated? The answer can be only a matter of speculation. Hybridization in fungi has been demonstrated, and the origin of new forms and races by mutation is generally accepted. The probabilities are that the different bean rust forms originated by one or the other of these methods, if not by both. Bean rust is autoecious and aecia have not been reported to occur under field conditions, but this circumstance does not necessarily preclude the possibility of their occurrence and their hybridization under natural conditions. On the other hand, no one has demonstrated that mutations have occurred in bean rust. It is probably safe to assume that the different physiologic races originated from a single ancestral line and that from it the new forms now known have developed either by hybridization or mutation. If these methods of origin are accepted, one is justified in concluding that new races will originate with increasing frequency in the future.

The distribution of the different physiologic races is shown in table 3. The collections are limited and a larger number from many more localities would be necessary before any general conclusions could be drawn respecting the distribution of the different races. Furthermore, it is believed that many more races could be discovered by more and wider collections. Foreign countries offer a fertile field from which to recruit new forms, and knowledge of such forms and the varieties of beans resistant to them is desirable in any comprehensive program for breeding rust-resistant varieties.

Bean rust epidemics are exceedingly sporadic except in a very few localities where they occur nearly every year. This indicates that rather exacting environmental conditions are probably required by the parasite.

Beans are grown in every section of the country either commercially or in the home garden, and in view of the facility with which the spores migrate their presence can be assumed. Yet epidemics are the exception rather than the rule, and there is no reason to assume that an outbreak will occur every year even though there is an abundance of spore material. Neither is there any reason to assume that if one race could start an epidemic all others could do the same thing under the same conditions, which would be equivalent to admitting that all

races are identical in every way except in the choice of the bean varieties they infect. They are presumably individualistic. Temperature, humidity, and other environmental conditions that are optimum for one form of rust may not necessarily be optimum for another. If this is true, one form of rust might initiate an epidemic while a contemporary race under identical conditions could not. Several physiologic races are usually obtained in a single collection, but generally one greatly predominates in number of spores over the others. The form that causes the epidemic is probably the one best adapted to the conditions prevailing at the opportune time, when there is a timely compatability between the host and the parasite.

In view of the large number of races of rust already known, what are the chances of developing resistant varieties? The difficulties increase with the increase in the number of specialized forms. A knowledge of the physiologic races themselves and the environmental factors that contribute to their behavior are prerequisites in any extensive program of breeding for rust resistance. The large number of commercial bean varieties on the market increases the difficulties of the problem. Most of these varieties hold a firm place in the trade and cannot be ignored. The susceptibility or resistance of most of the bean varieties has been determined for many though not all of the physiologic races. A careful study of table 2 (p. 724) will show that there are several varieties resistant to any one race of rust, and the proper selection of parents should ultimately yield productive rust-resistant progenies of the desired type. There are several varieties that are promising as parental material. Two of the differential hosts (Nos. 765 and 780) are resistant to many forms of rust. No. 765 is highly susceptible to only race 13, and No. 780 to only races 13, 14, and 16. Such varieties as the London Horticultural and Low's Champion also have promise.

The foreign field has not been thoroughly explored for resistant varieties and suitable parental stock. The material from foreign countries that has been examined by the writers was frequently composed of mixtures of many varieties and biotypes. Few of the varieties were pure lines and many were poor as to type and quality. Better progress is probably possible by making use of the varieties grown in the United States whose quality, type, and rust behavior are known.

SUMMARY

Twenty physiologic races of bean rust (*Uromyces phaseoli typica*) have been differentiated on the basis of their reaction to seven differential host varieties. The races were identified from bean material received or collected from many localities in the United States and from the Hawaiian Islands.

The grades of rust infection were arbitrarily established on a scale ranging from 0 to 10, immune varieties being graded 0, varieties with the highest degree of susceptibility, 10, and varieties with intermediate degrees of susceptibility being graded between these two extremes. Grades 3 to 5 or possibly 6 are regarded as commercially resistant. The degree of infection was found to be influenced by environmental conditions such as light, heat, and length of day. The sori were slow to develop and remained undersized when the inoculations were made during the cloudy and short days of the winter

months. A grade 1 infection in the winter months would often become a grade 2 or 3 on certain varieties if the inoculations were made in the spring or fall.

Necrotic lesions characteristic of resistance could be detected in about 3 days after inoculation on the under side of the leaf, while the flecks that develop later into large pustules were not evident before 5 days. Thus, evidence of resistance could be observed before there were any signs of infection on more susceptible hosts. The resistant necrotic lesions varied much in shape and size.

The susceptibility and resistance of a large number of commercial bean varieties were tested with races 1 to 14. A few varieties were highly resistant or immune to each race of rust, making it possible to obtain at least one parent variety that could be utilized in breeding for disease resistance.

Two or three physiologic races may be present in one locality during a single year. The number of spores of one usually greatly predominates over the others. These races do not necessarily recur in succeeding years in the same locality. They may be replaced by an entirely different race or races.

Urediospores can be kept in a viable condition for as long as 2 years if the leaves are dried for a few days at room temperature and thereafter stored at −20° C.

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RELATIONS OF NECTAR CONCENTRATION TO GROWTH OF ERWINIA AMYLOVORA AND FIRE BLIGHT INFECTION OF APPLE AND PEAR BLOSSOMS [1]

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INTRODUCTION

The epidemiology of the blossom-blight phase of fire blight of apple (*Malus sylvestris* Mill.) and pear (*Pyrus communis* L.) incited by *Erwinia amylovora* (Burr.) Winslow et al. is very variable. The reasons for this variability are not satisfactorily understood. In recent years attention has been directed to nectar concentration as a factor influencing blossom infection.

Beutler (*3*) [2] studied the influence of various factors on nectar concentration, and showed that the concentration of nectar sugars stands in close inverse relationship to relative humidity. Thomas and Ark (*10*) showed that fire blight infection of apple and pear blossoms is favored by low concentrations and hindered or prevented by high concentrations of nectar. They proposed that the increase in volume and reduction in concentration of nectar during humid weather bears an important relation to the incidence of fire blight. Further evidence bearing on the relation of nectar concentration to fire blight infection was presented by Hildebrand (*4*), Hildebrand and Phillips (*6*), and Ark (*1*). These experiments relating to fire blight were conducted chiefly in the laboratory with cut blossoms of apple or pear or with cultures of *Erwinia amylovora* in comparatively large amounts of media in test tubes. The present paper reports further studies designed to aid in interpreting relations of nectar concentration to variability in the epidemiology of blossom blight. Special attention was given to work with trees in bloom, either in the orchard or under partly controlled conditions in the greenhouse. These studies were interrupted when one of the writers was called to another post. The available results, though in some respects fragmentary, are reported herein.

Experiments were directed along three major lines, as follows: (1) Growth and survival of the bacteria in various concentrations of nectar; [3] (2) measurements of nectar concentrations under natural conditions in relation to time, temperature, and relative humidity; and (3) relation of nectar concentration to the initiation of blossom infection.

[1] Received for publication February 5, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 742.

[3] Natural and artificial nectars were used. Armbruster (*2*) gives a general review of literature on nectar. Beutler (*3*) reports occurrence of invert sugars and sucrose in apple nectar in the proportions by weight of 12.8 to 8.5. Thomas and Ark (*10*) report analyses of pear nectar showing relatively less sucrose. The artificial nectar used in the present work contained invert sugars and sucrose in the proportions reported by Beutler (*3*) for apple nectar, 6.4 parts by weight of dextrose, 6.4 of levulose, and 8.5 of sucrose being dissolved in the following weak nutrient solution: Asparagine, 0.1 percent; sodium chloride, 0.01 percent; dibasic potassium phosphate, 0.05 percent; magnesium sulfate, 0.02 percent; calcium chloride, a trace. This nutrient-sugar solution, hereinafter referred to as artificial nectar or artificial nectar solution, was adjusted to pH 7.0.

GROWTH AND SURVIVAL OF THE FIRE BLIGHT BACTERIA IN VARIOUS CONCENTRATIONS OF NECTAR

The influence of concentration of artificial nectar on the growth of fire blight bacteria in vitro was studied in Van Tieghem-cell cultures in which the amount of nectar and the number of bacteria approximated conditions encountered in nature. The cultures were prepared as follows: The nutrient solution in a large flask and the sugar mixture[4] in suitable amounts in test tubes were sterilized separately in the autoclave. Bacteria[5] from 1-day- to 2-day-old cultures were then introduced into the cooled flask in such numbers that a ¼₀₀-cc. drop of the complete medium would contain about 15 to 30 bacterial cells. Measured amounts of this nutrient solution were then added to the test tubes containing the sugars so that a series of seeded media was obtained containing, respectively, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, and 60 percent of sugars. In addition there were tubes that contained the weak nutrient solution with bacteria but without sugar, and others that contained the nutrient solution without bacteria or sugar. The concentration of the sugars in these media was checked by means of an Abbé refractometer and found to correspond to the desired percentage in each case. Hanging-drop cultures (¼₀₀ cc.) were made and placed in sterile Petri dishes. To prevent concentration of the solution through evaporation, the cover slips bearing the drops were sealed with vaseline all around the ring, except for a small opening to allow exchange of gases in the cell. For the same purpose a small amount of the solution (not seeded) from which the drop was taken was placed at the bottom of the cell and in the Petri dish. The Van Tieghem cells were prepared in triplicate for each nectar concentration and incubated for 6 days at about 20° C. The bacteria in the drops were counted at the end of the incubation period after fixing and staining.

The results showed that the bacteria multiplied considerably in drops containing no sugars and in those containing 1 to 10 percent of sugars, but they did not increase greatly in the drops containing 20 percent. No bacterial cells were seen in drops containing 30 percent or more of sugars, and no colonies appeared on nutrient-dextrose-agar plates when transfers from these drops were made. Growth increased sharply in the 1-percent drops and reached its optimum in the 2- to 4-percent drops, after which it diminished rapidly as the concentration of the sugars increased. The limiting sugar concentration for growth under the experimental conditions seemed to be between 20 and 30 percent. Some of the drops were kept for 14 more days, but no appreciable changes in the growth of the bacteria were noted. At this time the concentration of sugars in the drops was again measured with the refractometer and found to be about the same as at the beginning of the trials. The detailed data of this and two similar trials are given in table 1.

[4] See footnote 3, p. 733.

[5] Two isolates of *Erwinia amylovora* were used in these tests, one of medium pathogenicity and the other highly pathogenic. Both were progenies of single cells, isolated in hanging drops with microscopic control (*11*, *12*).

TABLE 1.—*Growth of Erwinia amylovora in drops of artificial nectar of various concentrations in Van Tieghem cells*

Percentage concentration of sugars in nectar	Bacteria per cubic centimeter[1] in—			
	First trial	Second trial	Third trial	Average
	Number	*Number*	*Number*	*Number*
0	7,000,000	23,000,000	48,000,000	26,000,000
1	60,000,000	125,000,000		92,500,000
2	112,000,000	130,000,000	800,000,000	347,333,333
3	112,000,000	102,000,000		107,000,000
4	79,000,000	58,000,000	580,000,000	239,000,000
5	18,000,000	28,000,000		23,000,000
6	1,440,000	22,000,000	16,000,000	13,146,666
7	840,000	22,000,000		11,420,000
8	173,000	6,000,000	6,000,000	4,057,666
9	17,000	2,000,000		1,008,500
10		2,000,000	3,000,000	2,500,000
20	7,200	0	18,000	12,600
30		0	0	0
40		0		0
50		0		0

[1] Number is approximate. The isolate used in trials 1 and 2 was of medium pathogenicity; that used in trial 3 was highly pathogenic, faster growing, and produced more abundant growth on several media. Trial 1 was made in 1936; trials 2 and 3 were made simultaneously in 1937. In estimating the number of bacteria per cubic centimeter both dead and living organisms were included.

Tests were made on the survival of the fire blight bacteria in drops of concentrated artificial nectar in Van Tieghem cells. The size of drops and rate of seeding were the same as in the experiments just reported. Transfers from these seeded drops were made at successive time intervals to drops of artificial nectar containing 1 percent of sugars, a concentration that favors growth. The results showed that the bacteria survived in the nectar containing 20 percent of sugars for 48 hours, 30 percent for 24 hours, and 40 and 50 percent for less than 24 hours.

Pear blossoms were inoculated with bacteria that had stood for 24, 48, and 72 hours, respectively, in test tubes partly filled with artificial nectar solution containing 40 percent of sugars. The turbidity of the bacterial suspensions corresponded to No. 2 of McFarland's nephelometer (*9*). Enough bacteria survived for 72 hours in nectar containing 40 percent of sugars to induce infection. However, this experiment differed greatly from the trials in Van Tieghem cells in the amount of media used and the concentration of the bacteria.

RELATION OF AMOUNT OF INOCULUM TO INITIATION OF BLOSSOM INFECTION

Hildebrand (*5*) obtained no infection of blossoms of dwarf pear trees when approximately 100 individual cells were placed singly on the stigmas, anthers, and nectaries. Under similar conditions, however, infection resulted in 1 out of 2 trials on dwarf apple trees when a single cell was transferred to the nectary. The relative humidity during these experiments was about 50 percent. The concentration of the nectar was not measured. In another trial he obtained infection in 9 out of 15 cases when single cells were placed on apple nectaries of excised flowers held in moist chambers in a room at about 24° C. In

similar tests with increased numbers of bacteria 100 percent infection was obtained from all inoculations in which 10 or more cells were used.

Greenhouse infection experiments were made by the present writers on the blossoms of potted dwarf Bartlett pear trees when the concentration of the nectar sugars was kept at about 1 to 5 percent. Such nectar concentrations were induced by keeping the trees in a moist chamber for several hours or overnight, after which the receptacle cups were usually found to be filled or overflowing with dilute nectar. Bacteria from a highly pathogenic culture grown 36 hours on nutrient dextrose agar slants were introduced into the receptacle cups in $\frac{1}{100}$-cc. drops of sterile distilled water standardized to contain approximately 10, 100, or 1,000 bacterial cells. Care was taken not to wound the blossoms. From 32 to 48 blossoms were used in each trial. The results were as follows, the first figure in each group showing the approximate number of bacteria in the inoculum and the second the percentage of blossoms infected: 10, 66; 100, 91; 1,000, 93. These data, which are in general agreement with those of Hildebrand (*5*), indicate that 10 bacteria constitute a highly efficient inoculum when introduced into the nectar under sufficiently favorable conditions.

MEASUREMENTS OF NECTAR CONCENTRATIONS UNDER NATURAL CONDITIONS

Since nectar concentration may be a limiting factor in blossom-blight infection, it seemed desirable to study the concentrations actually encountered under natural conditions in Wisconsin orchards. Measurements were made by means of an Abbé refractometer, with record of time of reading, temperature, and relative humidity. The nectar was collected from one or more blossoms, depending on the quantity in which it was available. Illustrative results are shown in tables 2 and 3.

It is obviously not to be assumed that these limited data can be taken as representative or as covering the range of conditions that may be encountered in this State. The weather conditions, however, were not unusual. It may be noted that no rainfall occurred on the days of observation, except light showers on June 5 and 8, 1937 (table 3). In a large majority of the readings the nectar concentrations were well above those at which the fire blight bacteria can multiply.

The evidence of diurnal fluctuations in the nectar concentration of apple blossoms at Madison in the period of May 15–17 and at Sturgeon Bay, May 24–28 (table 2) is of especial interest in relation to potentialities for fire blight infection. The early morning reading in each case showed a concentration favorable for infection, whereas the readings taken later in the day usually did not. None of the readings on pear nectar at Sturgeon Bay in the period May 30–June 11 (table 3), however, showed nectar concentration favorable for infection, except the one made after the rain of June 8.

A striking aspect of these data, meager though they are, is the strong predominance of nectar concentrations that would preclude multiplication of the fire-blight bacteria and be disadvantageous for their survival.

TABLE 2.—*Concentrations of pear and apple nectar under natural conditions, as related to time of day, temperature, and relative humidity, at Madison and Sturgeon Bay, Wis., 1936*

Location and kind of blossom	Date in May	Hour of measurement	Temperature	Relative humidity	Approximate concentration of sugars in nectar
Madison:			°F.	*Percent*	*Percent* [1]
Pear	12	7:30 a. m.	65	87	2
Do	12	9:00 a. m.	71	69	
Do	12	2:30 p. m.	72	73	
Apple	12	2:30 p. m.	72	73	
Do	12	6:00 p. m.	67	68	
Pear	12	6:00 p. m.	67	68	
Do	13	9:00 a. m.	57	72	
Do	14	11:00 a. m.	63	61	
Crab apple	14	6:00 p. m.	63	60	48
Apple	15	7:00 a. m.	53	55	5
Crab apple	15	6:00 p. m.	73	50	48
Apple	16	9:00 a. m.	70	70	5–10
Do	16	12:00 m.	81	42	
Do	16	6:00 p. m.	83	40	
Do	17	11:00 a. m.	75	62	5
Do	17	4:00 p. m.	75	60	[2] 40
Sturgeon Bay:					
Apple	24	7:00 a. m.			5–10
Do	24	12 m.			[2] 21
Do	24	4:30 p. m.	73	65	[2] 70
Do	27	7:30 a. m.	60	70	1–10
Do	27	12:00 m.	71	53	
Do	27	6:00 p. m.	62	58	
Do	28	7:30 a. m.	51	72	4
Do	28	12:00 m.	70	55	

[1] Where no reading is given the receptacle cups were dry or nearly dry.
[2] Measurements made on a small composite sample diluted with water, and percentage of sugars estimated.

TABLE 3.—*Concentrations of pear nectar under natural conditions, as related to time of day, temperature, and relative humidity, at Sturgeon Bay, Wis., 1937*

Date and hour of measurement	Temperature	Relative humidity	Concentration of sugars in nectar	Date and hour of measurement	Temperature	Relative humidity	Concentration of sugars in nectar
May 30:	°F.	*Percent*	*Percent* [1]	June 7:	°F.	*Percent*	*Percent* [1]
7:00 p. m.	70	68	36	7:00 a. m.	55	71	
May 31:				11:00 a. m.	64	56	
8:00 a. m.	82	69	36	8:00 p. m.	52	75	
12:00 m.	87	57	61	9:00 p. m.	52	[3] 58	
4:30 p. m.	86	57	59	June 8:			
8:00 p. m.	70	67	35	7:30 a. m.	54	88	[2]
June 1:				9:00 a. m.	55	76	3
8:00 a. m.	50	87	36	3:00 p. m.	60	58	
12:00 m.	59	63	50	6:00 p. m.	55	65	
12:45 p. m.	61	62	52	June 9:			
6:15 p. m.	58	83	38	7:45 a. m.	55	65	
June 3:				9:00 a. m.	60	49	
8:00 a. m.	62	70		2:15 p. m.	68	47	
6:00 p. m.	60	73		June 10:			
June 4:				11:30 a. m.	74	45	
10:30 a. m.	64	75		5:30 p. m.	69	64	
June 5:				June 11:			
6:30 a. m.			[2]	12:00 m.	83	46	
5:30 p. m.	60	97					
June 6:							
1:00 p. m.	66	53					
4:00 p. m.	59	68					

[1] Where no reading is given the receptacle cups were dry or nearly dry, unless otherwise noted.
[2] Light shower. No reading made.
[3] Wind.

RELATION OF NECTAR CONCENTRATION TO BLOSSOM INFECTION

It was sought to check and supplement the evidence thus far discussed by blossom-infection experiments under partly controlled conditions in which the relations of nectar concentration were studied.

Field trials in which the blossoms were inoculated by means of a small camel's-hair inoculator with bacteria suspended in artificial nectar solutions of various concentrations were made at Madison and Sturgeon Bay in orchards in which no natural occurrence of fire blight was observed. The inoculated blossom clusters and controls were bagged during the incubation period. The results, which are given in tables 4 and 5, indicate that infection occurred freely when the bacteria were introduced in low concentrations of nectar, but that it was lacking or sparse when they were introduced in the highly concentrated artificial nectar. In this connection it should be noted that in at least one trial with concentrated nectar infection occurred long after the usual incubation period (footnote 3, table 4). It is thought that such infection may have occurred only after the nectar had become diluted through a natural process. It is likely that under field conditions the concentration of nectar may change considerably in a short time and thus facilitate, retard, or prevent infection after inoculation has taken place. This question was further investigated in experiments reported in the following paragraphs.

TABLE 4.—*Concentration of artificial nectar in which the bacteria were suspended in relation to blight infection of pear and apple blossoms, at Madison, Wis., 1936*

Kind of blossom	Concentration of sugars in the bacterial suspension	Treatment of blossoms [1]	Time inoculated and atmospheric conditions				Blossoms—	
			Date in May	Approximate hour	Temperature	Relative humidity	Treated	Diseased [2]
	Percent				*° F.*	*Percent*	*Number*	*Percent*
Pear	0	Inoculated without wounding.	12	7:30 a.m.	65	87	120	84
	0	Inoculated by puncturing	12	7:30 a.m.	65	87	14	100
		Water drop without bacteria placed in receptacle cup.	12	7:30 a.m.	65	87	15	0
	1	Inoculated without wounding.	12	6:00 p.m.	67	68	30	50
	40	do	12	6:00 p.m.	67	68	18	[3] 0
	0	do	13	9:00 a.m.	57	72	30	50
	0	Inoculated by puncturing	13	9:00 a.m.	57	72	10	100
	40	Inoculated without wounding.	13	9:00 a.m.	57	72	20	0
	40	Inoculated by puncturing	13	9:00 a.m.	57	72	10	100
		Blossoms not treated, same branch.[4]	13	9:00 a.m.	57	72	50	0
	0	Inoculated without wounding.	14	11:00 a.m.	63	61	24	79
	1	do	14	4:00 p.m.	65	55	25	68

[1] Inoculations without wounding were made by placing a small drop of bacterial suspension in the receptacle cup by means of a small camel's-hair-brush inoculator.

[2] Records on disease development were taken about a week after treatment.

[3] Most of the inoculated blossoms were found diseased at a later inspection. Infection may have started later, after conditions in the receptacle cup became more favorable.

[4] On the same branch as puncture inoculations of the same date.

TABLE 4.—*Concentration of artificial nectar in which the bacteria were suspended in relation to blight infection of pear and apple blossoms, at Madison, Wis., 1936*—Continued

Kind of blossom	Concentration of sugars in the bacterial suspension	Treatment of blossoms	Time inoculated and atmospheric conditions				Blossoms—	
			Date in May	Approximate hour	Temperature	Relative humidity	Treated	Diseased
	Percent				°*F.*	*Percent*	*Number*	*Percent*
Apple	1	Inoculated without wounding.	14	4:00 p.m.	65	55	50	82
	1	Inoculated by puncturing	14	4:00 p.m.	65	55	25	100
	.....	Blossoms not treated, same branch.[4]	14	4:00 p.m.	65	55	73	0
	1	Inoculated without wounding.	16	11:00 a.m.	77	47	25	76
	5	do	16	11:00 a.m.	77	47	80	77
	40	do	16	11:00 a.m.	77	47	24	0
	40	Inoculated by puncturing	16	11:00 a.m.	77	47	15	100
	.....	Blossoms not treated, same branch.[4]	16	11:00 a.m.	77	47	23	0
	0	Inoculated without wounding.	17	10:00 a.m.	77	65	25	24
	0	Inoculated by puncturing	17	10:00 a.m.	77	65	27	100
	2	Inoculated without wounding.	17	10:30 a.m.	76	63	35	51
	4	do	17	10:30 a.m.	76	63	25	44
	6	do	17	11:00 a.m.	75	62	24	0
	8	do	17	11:00 a.m.	75	62	25	0
	20	do	17	11:30 a.m.	75	62	25	0
	40	do	17	11:30 a.m.	75	62	25	0
	40	Inoculated by puncturing	17	12:00 m.	75	62	26	100
	.....	Water drop without bacteria placed in receptacle cup.	17	12:00 m.	75	62	32	0

[4] On the same branch as puncture inoculations of the same date.

TABLE 5.—*Concentration of artificial nectar in which the bacteria were suspended in relation to blight infection of apple blossoms, at Sturgeon Bay, Wis., 1936*

Percentage concentration of sugars in the bacterial suspension [1]	Treatment of blossoms [2]	Blossoms—		Bacterial exudate present
		Treated	Diseased	
		Number	*Percent*	
0	Inoculated without wounding	28	28	Yes.
0	Inoculated by puncturing	7	100	Yes.
2	Inoculated without wounding	28	78	Yes.
2	Inoculated by puncturing	8	87	Yes.
4	Inoculated without wounding	17	64	Yes.
6	do	24	70	Yes.
10	do	45	46	Yes.
20	do	33	30	No.
20	Inoculated by puncturing	8	100	No.
40	Inoculated without wounding	27	7	Yes.[3]
40	Inoculated by puncturing	6	83	No.
	Water drop without bacteria placed in receptacle cup.	42	0	
	Blossoms bagged but not treated	48	0	

[1] A 0.3-percent supplement of sodium ricinoleate was added to each artificial nectar solution for the purpose of decreasing the surface tension of the solution.

[2] See footnote 1, table 4.

[3] The time of infection of these blossoms questionable.

In a greenhouse trial the blossoms of potted pear trees were inoculated with bacteria suspended in artificial nectar ranging in sugar concentration from 1 to 40 percent. Some of the trees received a 12- to 16-hour treatment in a moist chamber (7) at 20° to 22° C. immediately after inoculation, while others were left in the greenhouse without such treatment. One hundred percent infection occurred in blossoms inoculated with artificial nectar suspension containing 1 percent of sugars, whether the trees were given the moist treatment or not. Like results were obtained with blossoms inoculated with a nectar suspension containing 10 percent of sugars, with a moist treatment following inoculation. Considerable reduction in infection occurred in blossoms inoculated with this same nectar suspension with no treatment following inoculation, and in blossoms inoculated with a nectar suspension containing 40 percent of sugars with a moist treatment following inoculation. No infection occurred in blossoms inoculated with the suspension containing 40 percent of sugars but given no moist treatment after inoculation. In these trials no records were taken of the concentration of nectar in the blossoms during the incubation period. Such records seem necessary in order better to interpret some of the results obtained. The trials reported in the following paragraph afford such data.

Three successive trials were made in the field at intervals of several days, in which pear blossoms of an unknown variety were inoculated with fire blight bacteria suspended in water and in artificial nectar solutions containing 10 and 40 percent of sugars, respectively (table 6). On 3 successive mornings following inoculation, records were taken of the concentration of the mixed artificial and natural nectar and of the natural nectar of untreated blossoms. In trials 1 and 2 infection occurred only in blossoms inoculated with bacteria suspended in water. The sugar concentration of the mixed artificial and natural nectar and also of the natural nectar for the first 3 days after inoculation stood at 52 percent or higher. In some cases the nectar was so concentrated that no measurable samples were obtainable. During these 3 days the relative humidity was above 70 percent and no rain or heavy dew occurred. In the third trial, however, 100 percent infection occurred at the end of the incubation period in all the inoculated blossoms. The concentration of the mixed artificial and natural nectar and the natural nectar during the first 2 days following inoculation was relatively low, however, varying from 3 to 15 percent. During the 2 days light showers fell at intervals, the skies were clouded, the temperature was relatively low, and the relative humidity was near the saturation point for several hours at a time. Seven other similar trials were made during the same season at Sturgeon Bay with similar results.

TABLE 6.—*Relation of nectar concentration to blight infection of pear blossoms in the orchard, at Madison, Wis., 1937*

Trial No.	Concentration of sugars in artificial nectar in which the inoculum was suspended	Concentration of sugars in the natural nectar of controls at indicated time after inoculation			Concentration of sugars in the mixed artificial and natural nectar at indicated time after inoculation			Blossoms—	
		1 day	2 days	3 days	1 day	2 days	3 days	Inoculated	Diseased
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
1	0	[1]	[2]	[2]	[1]	[3]	[2]	48	62
	10	[1]	[2]	[2]	[1]	[2]	[2]	45	0
	40	[1]	[3]	[3]	52	56	68	92	0
2	0	[3]	[3]	[3]	[2]	[2]	[2]	60	63
	10	[3]	[3]	[3]	[3]	[2]	[2]	45	0
	40	[2]	[3]	[3]	62	60	66	68	0
3	0	[3] 3	15	[3]	2	11	[2]	65	100
	10	3	15	[3]	6	13	[1]	64	100
	40	3	15	[2]	12	14	42	120	100

[1] Sugar concentration not measurable.
[2] Cups dry.
[3] Rain fell a few hours after inoculation. Humidity continued high (90 to 99 percent) throughout the following day.

DISCUSSION

When the bacteria were placed in small droplets of artificial nectar in Van Tieghem cells in the method used in the present work, they tended to be limited in growth at somewhat lower sugar concentrations and to survive for shorter periods than in the test-tube experiments reported in earlier work (*1, 6, 10*) or herein. Growth of the bacteria in artificial nectar and infection of unwounded blossoms inoculated in the nectar occurred freely under the conditions of these experiments only when the sugar concentrations were in the lower range encountered in natural nectar. Sharp inhibition or prevention of growth and infection occurred at the lower intermediate concentrations. There was usually no growth or infection at the medium and higher concentrations, which predominated in the orchard observations.

The data thus far available shed considerable light on the relations of sugar concentration to the growth of *Erwinia amylovora* in vitro and to blossom-blight infection following artificial inoculation. They show beyond doubt that nectar concentration can limit blossom-blight infection, but the detailed manner and extent of its influence on epidemiology seem still to be obscure in many respects. Are the results obtained in a limited number of experiments under artificial conditions a sufficient guide to the interpretation of what happens in the wide ranges of nature? Do we know what to expect when the bacteria are introduced into the nectar by insects? Will they be transmitted freely by nectar-sipping insects from one blossom to another when the nectar concentration is high? If so, will they survive and initiate infection when the nectar becomes sufficiently dilute? For what time and through what range of natural conditions can the bacteria thus survive in the concentrated nectar and still be able to incite infection? Do such insect-deposited bacteria commonly lie in wait in the concentrated nectar of apple and pear blossoms, ready to initiate infection as soon as a favorable dilution permits? Or

are there sharp limitations on insect transmission and survival of the bacteria in nectar? If so, what is their nature? Adequately controlled experiments involving natural transmission of the bacteria seem necessary for the elucidation of such questions. A beginning of studies on the experimental transmission of fire blight by bees and its relation to nectar concentration of apple and pear blossoms is reported elsewhere in this Journal (*8*).

SUMMARY

The optimal concentration of sugars for growth of the fire blight pathogen, *Erwinia amylovora*, in ¼₀₀-cc. drops of artificial nectar in Van Tieghem cells was 2–4 percent. Growth rapidly decreased with increased sugar concentration, none occurring at 30 percent. The bacteria survived for 48 hours in similar drops containing 20 percent of sugars, 24 hours in 30 percent, and less than 24 hours in 40 or 50 percent. When tubes of artificial nectar containing 40 percent of sugars were heavily seeded, the bacteria survived for 72 hours and incited infection when placed in pear blossoms.

Approximately 10 bacteria in a ¼₀₀-cc. water drop constituted an effective inoculum when placed in dilute nectar of pear blossoms.

Abbé refractometer readings are reported for nectar of pear (*Pyrus communis*), apple (*Malus sylvestris*), and crab apple (*Malus coronaria*) collected under various conditions in nature, with record of time, temperature, and relative humidity.

Infection of unwounded pear or apple blossoms inoculated by placing small droplets of bacterial suspension in the nectar occurred freely only when the sugar concentrations were in the lower range encountered in natural nectar. Sharp inhibition or prevention of infection occurred at the lower intermediate concentrations. There was usually no infection at the medium or higher concentrations, which predominated in the orchard readings. However, infection occurred if the concentrated nectar was sufficiently diluted soon enough after inoculation.

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TRANSMISSION OF FIRE BLIGHT BY BEES AND ITS RELATION TO NECTAR CONCENTRATION OF APPLE AND PEAR BLOSSOMS[1]

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INTRODUCTION

The pioneer investigations of Waite (*17, 18, 19*)[3] and later experiments and observations by many others have established beyond doubt that the honeybee and some other insects are capable of transmitting the blossom blight of apple (*Malus sylvestris* Mill.) and pear (*Pyrus communis* L.) incited by *Erwinia amylovora* (Burr.) Winslow et al. For many years after this discovery insects were generally thought to be the only important agents for transmission of blossom blight. Later investigations (e. g., *2, 3, 4, 7, 10, 16*), however, have shown that meteoric water is an important factor in its spread and that under some conditions minute aerial strands of bacterial exudate may be disseminated by wind. The relative importance of insect and water transmission, which seems to vary greatly with conditions, is subject to considerable difference of opinion. Since blossom blight is one of the most important phases of the fire blight problem, a better understanding of its epidemiology is highly desirable.

Though it is generally accepted that bees can transmit blossom blight, comparatively little experimental work has been done on the details of this transmission or on the factors that favor or limit it. It has been the purpose of the present work to contribute to a reexamination of these aspects of the problem in the light of recent information, especially in their relation to nectar concentration. These studies, which were pursued in the spring of 1936 and 1937, were unavoidably interrupted when one of the authors was called to another post. The available results are reported herein. A companion study on nectar concentration in relation to the growth of *Erwinia amylovora* and infection of blossoms following artificial inoculation is reported elsewhere in this Journal (*8*).

Literature on the transmission of fire blight has been reviewed by Parker (*11*) and others. Consequently, only papers that seem especially pertinent to the present work are cited herein.

DIRECT TRANSMISSION FROM CONTAMINATED TO UNCONTAMINATED BLOSSOMS

An objective of the following experiments was development of methods whereby transmission of fire blight from one blossom to another by honeybees (*Apis mellifera* L.) could be studied experimentally under adequate control. Some needs for specific information

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[3] Italic numbers in parentheses refer to Literature Cited, p. 752.

concerning such transmission in relation to the epidemiology of blossom blight have been discussed elsewhere (*8*).

Experiments were performed in three ways: (1) Individual marked bees of an uncontaminated nucleus hive in a large cloth cage in the greenhouse successively visited inoculated [4] and uncontaminated blossoms of potted trees; (2) bees of an uncontaminated nucleus hive in a large cloth cage in the greenhouse freely visited two potted trees, one with inoculated blossoms and the other with uncontaminated ones; and (3) an uncontaminated bee, handled in a specially designed wire cage, visited inoculated blossoms and then uncontaminated ones in the greenhouse or the orchard.

Evidence that the bees were not contaminated with the fire blight bacteria at the beginning of the experiments is based on the following precautions and tests. The bees of the nucleus hives were brought from Louisiana in early spring before they had opportunity to leave the hive. If the hive had been contaminated in the preceding year, the evidence is strongly against the possibility that the bacteria would have overwintered in it (*6, 11, 12, 14, 15*). When the hives had been placed in the cloth cages, the bees were allowed on 2 successive days to visit uncontaminated blossoms of potted apple and pear trees that had been held in a moist chamber to bring the nectar to a dilution favorable for infection. Each individual bee used in experiments with the small wire cage was allowed to work on a few uncontaminated blossoms before it was employed in the subsequent transmission experiments. In all these control experiments the bees were seen to introduce the glossa into the receptacle cup and remain in position long enough to indicate that they were sipping nectar. Throughout all the experiments on transmission, no indication was found that the bees were contaminated before they were permitted to visit the inoculated or diseased blossoms.

Some typical experiments on transmission are described below as illustrative of the methods and results.

About 50 blossoms of a 3-year-old dwarf Bartlett pear tree in the greenhouse were inoculated in the nectar by means of a small camel's hair inoculator at about 6 p. m. The tree had previously been held for a few hours in a moist chamber (*9*) with the curtains wet but spray not running, in order to bring the nectar to a concentration low enough to favor infection.[5] After inoculation it was kept overnight in another moist chamber. On the following morning measurements of nectar by means of an Abbé refractometer showed that the sugar concentration varied from 3 to 5 percent, whether the blossoms had been inoculated or not. Platings were made from inoculated and uninoculated blossoms at 3 time intervals that day. Fire blight bacteria, which subsequently caused typical infection in inoculation tests, were isolated in all trials from inoculated blossoms, but in no case from uninoculated ones. On the morning after inoculation, this tree, with a like one that had received similar moist treatment but no inoculation, was placed in a cloth cage. Each tree was protected by mosquito netting, so that

[4] Unless otherwise stated all inoculations were made on the preceding day by introducing a drop of about 1/400 cc. of bacterial suspension into the receptacle cup by means of a camel's hair inoculator, with care not to wound the host tissue.

[5] Unless otherwise stated all potted trees, before and after being used in the transmission experiments, were placed overnight in the moist chamber at about 20° C., with the curtains wet but the water not running. The inoculated and the uncontaminated trees were kept in separate chambers. At the end of a moist treatment, the nectar in the blossoms was usually abundant, and contained 3 to 5 percent of sugars. On keeping the trees in the greenhouse for 1 hour at about 24°, the concentration of nectar sugars usually rose to 10 or 12 percent.

individual blossom clusters could be exposed to visitation by bees or covered at will. An uncontaminated nucleus hive of bees was then placed in the cage. An inoculated cluster was exposed, with the aim of having one bee sip nectar from the blossoms.[6] Most of the bees flew about above the trees but only a few actually approached the exposed cluster. As soon as one alighted on an exposed blossom it was marked on the thorax with a droplet of specially prepared aniline dye and no other bees were permitted to touch this cluster. This bee was allowed to work on the inoculated blossoms until it had a good chance to become contaminated but not long enough to get its fill of nectar. The inoculated cluster was then covered and one on the uninoculated tree was exposed. The marked bee, without returning to the hive, alighted on the blossoms and worked on each of them, at times returning to a blossom it had already sipped from before going to another it had not yet visited. Each blossom the bee touched was marked, and the order of visitation was recorded. After the bee had visited all the blossoms, it was caught and the cluster was covered. The glossa was cut off with sterile instruments and its apical part dipped successively into the nectar of 10 blossoms of a third available tree having abundant nectar of a concentration favorable for infection. The glossa and the honey stomach were crushed and plated. The three trees were then incubated overnight in the moist chamber at about 20° C., with the curtains wet but the water shut off. On the following morning the blossoms of all the trees were found to contain abundant nectar. The trees were then further incubated in the greenhouse for 7 days at about 22° to 24°, after which the results were taken. The trees were kept under observation for another month.

The results of this experiment show that the bee transmitted the disease from the inoculated blossoms to 2 of the 4 uncontaminated blossoms it visited. No disease developed in the 10 blossoms that were touched with the bee's glossa. The glossa, however, yielded a few fire blight colonies that were subsequently shown by inoculation to be pathogenic. The honey stomach yielded a great number of micro-organisms, none of which resembled the fire blight pathogen. Most of the blossoms inoculated with the camel's hair inoculator showed macroscopic symptoms of blight within 4 days. Blossoms that were not inoculated did not show any disease. The experiment just described was performed four more times under similar conditions. Only one of these trials gave positive results.[7]

Transmission was also accomplished in two trials of another type, in which an inoculated and an uninoculated tree were kept together in a cloth cage for 5 hours, the bees of an uncontaminated hive freely visiting the blossoms of both trees. In the first trial 32 of the 61 blossoms of a dwarf Bartlett pear tree (uninoculated when placed in the cage) became diseased within a week after the bees' visitation, and in the second 46 of the 87 blossoms on a Seckel pear tree (likewise uninoculated when placed in the cage) blighted. The disease was therefore transmitted to 52 percent of the blossoms of these two trees.

[6] Bees may visit blossoms to collect pollen or reconnoiter without sipping nectar. In these experiments they were regarded as sipping nectar when they extended the glossa into the receptacle cup and remained in position for a distinctly longer period than would be required for reconnoitering.

[7] The amount and concentration of nectra changed rapidly after the trees were taken out of the moist chamber. For instance, in one case the concentration of nectar sugars rose from 3.5 percent to 11 percent in 50 minutes. In a few hours the volume of nectar was so diminished that measurable samples were not obtainable. These changes in the amount and concentration of nectar during the course of an experiment may have influenced the amount of infection (*8*), notwithstanding the fact that the moist treatment of the plants after visitation by the bees induced nectar concentrations favorable for infection.

Most of the tests on direct transmission of blossom blight by selected individual bees were made with the use of a small wire bee cage or trap (fig. 1). The chief advantage of this cage is that it permits keeping a particular bee with known history through several operations in association with any selected clusters as long as the bee remains alive and active. The cage, made of 16-mesh galvanized wire screen, is about 10 inches long and 4½ inches in width and height. It consists of two detachable halves connected with hinges on one side and with a hook on the opposite side. Two sliding doors, each placed near the outer end of one of the hinged sections, cut off small compartments (fig. 1, *a* and *c*). A bee is easily caught with this cage and confined in the central large compartment (fig. 1, *b*). Then

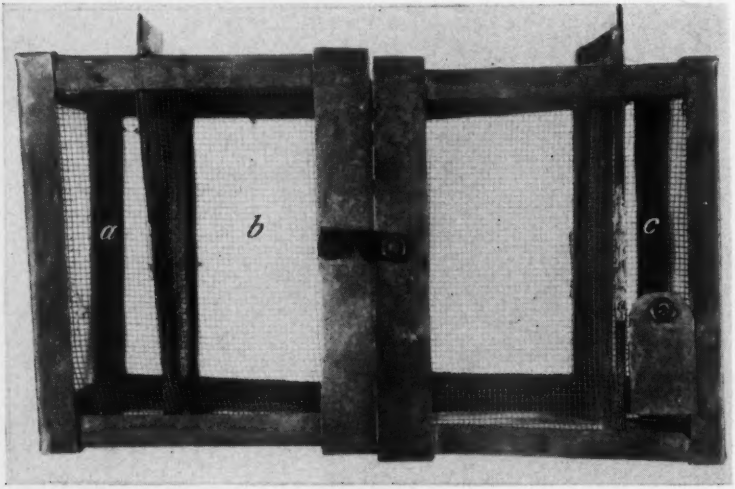

FIGURE 1.—Wire bee cage used in the inoculation experiments: *a* and *c*, Small outer compartments; *b*, large central compartment.

one of the trap doors is lifted and the insect is confined in the small outer compartment. Later the central compartment is opened, placed about a blossom cluster, then closed and hooked. The trap door is lifted, and the bee is allowed to visit the blossom cluster. After it has worked on the blossoms sufficiently, it is driven back into the small compartment and the sliding door is closed. The same bee is then used again on other blossom clusters, carried safely from one orchard to another, kept overnight, or handled otherwise according to the requirements of the experiment.

Transmission experiments with the wire bee cage included the following steps:

1. In an orchard in which no naturally occurring fire blight had been found, 10 blossom clusters of apple or pear were bagged separately, 2 of which were inoculated with the fire blight organism suspended in pure water or in artificial nectar[8] of various concentrations, as desired.

[8] The artificial nectar used contained invert sugars and sucrose in the proportions reported by Beutler (*1*) for apple nectar—6.4 parts by weight of dextrose, 6.4 of levulose, and 8.5 of sucrose being dissolved in the following weak nutrient solution: Asparagine, 0.1 percent; sodium chloride, 0.01 percent; dibasic potassium phosphate, 0.05 percent; magnesium sulfate, 0.02 percent; calcium chloride, a trace. The solution was adjusted to approximately pH 7.0. The details of preparation are reported elsewhere (*8*).

2. About 1 to 3 days later, a bee was caught in the sterilized cage and allowed to sip nectar from two of the uninoculated clusters. The total number of blossoms worked on was noted.
3. As soon as the cage could be shifted into position, the same bee was allowed to sip nectar from an inoculated cluster.
4. After a similar brief interval, the same bee was allowed to work on four of the uninoculated blossom clusters.
5. The bee then was decapitated, its glossa and honey stomach plated, and the pathogenicity of the recovered bacteria tested.

In some special trials the concentration of the nectar in the blossoms was measured just before or just after the bee's visit.

By using the technique just described, in some cases omitting steps 1 and 5, 32 transmission tests were made in the greenhouse or the orchard at Madison in 1936. Ten of these gave positive results; i. e., the bee transmitted the disease from an inoculated to an uncontaminated blossom.

The transmission tests were continued during the same season at Sturgeon Bay, Wis., where the blooming season is later than at Madison. In all cases the transmission was attempted with single bees, trapped in the wire cage. Of the 26 individual tests, 7 gave positive results, 13 negative, and 6 doubtful. Of the tests that gave positive results, 5 were made when the receptacle cups were moist or wetted and only 2 when they were apparently dry. None of the 13 tests that gave negative results were made when the receptacle cups were moist or wetted.

LENGTH OF TIME AFTER INOCULATION THAT BLOSSOMS ATTRACT BEES

One experiment was performed to gain evidence on the length of time after inoculation that blossoms will attract bees and serve as sources of contamination. It consisted in placing with an uncontaminated hive in the cloth cage two different Bartlett pear trees each day, one inoculated and the other not. The inoculated tree introduced on the first day had been inoculated 5 days; that on the second, 4; the third, 3; the fourth, 2. The results showed that under the conditions of the experiment the bees could transmit the disease to healthy blossoms from diseased blossoms that had been inoculated for 5 days. The diseased blossoms on the tree introduced 5 days after inoculation were already wilted and light brown in color. Three bees on more than 5 occasions touched these diseased blossoms with the glossa, then moved to the healthy blossoms. It was evident, however, that the healthy blossoms attracted more bees than the diseased ones and that the bees lingered longer on healthy than on diseased blossoms. The tree that was uninoculated when placed in the cage with the tree inoculated for 5 days had 46 blossoms, 29 of which were found diseased 10 days after the bees' visit.

CONCENTRATION OF NECTAR IN BLOSSOMS AT THE TIME OF THE BEE'S VISIT IN RELATION TO TRANSMISSION

In a greenhouse trial (table 1) some potted Bartlett pear trees were given a treatment in the moist chamber that brought the sugar concentration of their nectar within a range of 3 to 8 percent. They were then placed in a cloth cage and subjected to visitation by contaminated bees with similar trees that, having received no moist treatment, had

nectar with a sugar concentration of 45 percent. After removal from the cage the trees with low nectar concentration received a second moist treatment, whereas the others did not. Thirty-eight percent of the visited blossoms with the lower nectar concentration and none of those with the higher blighted.

TABLE 1.—*Nectar concentration in blossoms of 2-year-old Bartlett pear trees in relation to transmission of fire blight by contaminated honeybees* [1]

Bee No.	Moist treatment of blossoms before or after bee's visit [2]	Concentration of sugars in nectar before bee's visit [3]	Blossoms visited by bee	Blossoms diseased	
		Percent	*Number*	*Number*	*Percent*
1	Treated before and after	3, 4, 8	3	2	
1	do	3, 4, 8	5	2	
2	do	3, 4, 8	3	0	38
2	do	2, 3	4	1	
2	do	2, 3	2	2	
3	do	4, 5	4	1	
4	No moist treatment	45 or higher	4	0	
1	do	45 or higher	5	0	
4	do	45 or higher	5	0	0
5	do	(4)	3	0	
6	do	(4)	4	0	

[1] The bees had just been contaminated by sipping nectar from blossoms artificially inoculated on the preceding day. The experiments were performed in the greenhouse at 20° to 24° C.

[2] A bee visited from 2 to 5 blossoms of a cluster.

[3] Values are for individual samples. The concentration of nectar rose in some of the blossoms to 15 percent during the time the bee worked and before the tree was put back into the moist chamber.

[4] Receptacle cups dry.

In a field trial it was aimed to control in part the nectar concentration of the blossoms from which the bees obtained inoculum, as well as of those to which they were to carry it. In a pear orchard in which no naturally occurring blight had been found, blossoms were inoculated on various days with fire blight bacteria suspended in artificial nectar solutions with sugar concentrations varying from a trace to 40 percent. At the same time small drops of artificial nectar of the same range of concentration, but containing no bacteria, were placed in the receptacle cups of uncontaminated blossoms. Some of the treated blossoms were bagged in an attempt to check the rapid increase of nectar concentration. On the following day individual uncontaminated bees were allowed to sip nectar, first from some of the inoculated blossoms, then from uninoculated ones containing the artificial nectar drops. Shortly before or after the bees visited the blossoms, the concentration of the nectar in these and other blossoms was measured. It was found in most cases that the concentration had undergone changes. Some of the drops that originally had contained a trace or 1 percent of nectar sugars were later found to contain as high as 10 or 12 percent. Likewise, blossoms that originally contained 40 percent of nectar sugars were found to have 70 or 75 percent. The results of these trials, which are summarized in table 2, show that when a bee worked on inoculated blossoms with nectar containing 2 to 12 percent sugars and then on uncontaminated ones with nectar containing 0 to 35 percent sugars, 49 percent of the latter group blighted. A higher percentage of infection might have resulted if the concentration of nectar in some of the blossoms had not risen so high. On the other hand, no infection resulted when the bees first worked on blossoms with nectar containing, respectively, 10–14, 42–56, and 48–75 percent sugars and then on others with nectar containing, respectively, 10–18, 44–47, and 46–70 percent. Likewise, bees that

worked on inoculated blossoms with apparently dry receptacle cups and then on uncontaminated ones with dry receptacle cups did not transmit the disease.

TABLE 2.—*Concentration of pear nectar in relation to transmission of blossom blight by honeybees, Sturgeon Bay, Wis., 1937* [1]

Range of concentration of sugars in nectar of inoculated blossoms from which bees sipped	Range of concentration of sugars in nectar of healthy blossoms from which contaminated bees sipped	Bagging of blossoms after bees' visit	Bees used	Blossoms	
				Used	Diseased
Percent	*Percent*		*Number*	*Number*	*Percent*
2- 8	0-10	Bagged	12	50	56
3-12	0-35	Not bagged	8	42	40
10-14	10-18	Bagged	6	22	0
42-56	44-47	do	10	34	0
48-75	46-70	Not bagged	9	36	0
[2]	[2]	do	18	52	0

[1] Wire cages were used for controlling the bees and the experiments were performed on orchard trees.
[2] Receptacle cups dry.

DISCUSSION

It is recognized that some degree of artificiality may attend all experiments with bees handled in captivity, and that results from such work are reliable only in proportion to the adequacy with which they are observed and controlled.

Transmission of the disease from one blossom to another was demonstrated by each of the three methods tried, and each method may be useful. However, work with individual bees greatly facilitates adequate observations and controls. Use of the bee cage substantially increases the range and flexibility of experimentation with individual bees.

The large number of instances in which blossoms did not blight after visitation by a contaminated bee indicates that there are important limitations on the efficiency of this insect in transmitting the disease. Indeed, if this were not the case, it would be very difficult to understand how our apple and pear culture could continue, in view of the great number and activity of bees.

The results of these experiments on transmission of blossom blight by bees indicate that nectar concentration is a very important factor limiting this mode of transmission. They are in general accord with the results of studies (*5*, *8*, *13*, *14*) of nectar concentration in relation to fire blight infection initiated by artificial inoculation. However, in many cases in which the nectar was at a favorable concentration, little or no infection occurred after contaminated bees had sipped from it. It is, therefore, apparent that other factors besides nectar concentration are important in limiting blossom-blight transmission by bees. An experimental study of such factors lies beyond the scope of the present paper.

The need for information on factors favoring or limiting blossom-blight infection under conditions of natural transmission has been discussed elsewhere (*8*). The present investigation was interrupted soon after experimental methods for such work had been developed. Further studies under various conditions are needed. It would seem especially desirable to perform additional greenhouse and orchard

experiments in which contaminated bees visit uncontaminated blossoms containing nectar too concentrated to permit infection. The time during which bacteria thus deposited will live and the range of conditions they will tolerate without losing the capability to infect when favorable conditions occur are vitally important considerations in relation to the epidemiology and control of the disease. While work with artificial inoculation is very valuable in helping to define and interpret problems relating to blossom-blight transmission by bees and other insects, further experimental work on transmission by the insects themselves seems essential to an adequate understanding of their role in disseminating the disease.

SUMMARY

Transmission by honeybees of fire blight of apple (*Malus sylvestris*) and pear (*Pyrus communis*), incited by *Erwinia amylovora*, was studied by three experimental methods. The most flexible and convenient one employed individual bees handled in a specially designed wire cage.

Bees were attracted to blighting blossoms that had been inoculated 5 days before, and transmitted the disease to healthy blossoms.

In greenhouse and orchard experiments contaminated bees freely transmitted blight to healthy blossoms when the sugar concentration of the nectar was in the lower range encountered in nature, but not when it was in the medium or higher range.

In the experiments reported herein, nectar concentration was an important factor in limiting blossom-blight transmission by bees. However, in many cases in which the nectar was at a favorable concentration, little or no infection occurred after contaminated bees had sipped from it. It is apparent that other factors in addition to nectar concentration are important in limiting blossom-blight transmission by bees.

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